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(54)【発明の名称】　ペプチド及びその用途

(57)【要約】　（修正有）

【解決手段】　配列番号１のアミノ酸配列から成るペプ＊チド等。

配列番号１

Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser
1　5　10

【効果】　ペプチドはスギ花粉アレルゲンに特異的なイムノグロブリンＥ抗体に実質的に反応しないので、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起こすことなく、スギ花粉アレルゲンに特異的なＴ細胞を活性化できる。

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【特許請求の範囲】

【請求項１】　配列番号１のアミノ酸配列から成るペプチド。

【請求項２】　配列番号２のアミノ酸配列から成るペプチド。

【請求項３】　配列番号３のアミノ酸配列から成るペプチド。

【請求項４】　配列番号３のアミノ酸配列を含むことから成るペプチド。

【請求項５】　配列番号４のアミノ酸配列から成るペプチド。

【請求項６】　配列番号５のアミノ酸配列から成るペプチド。

【請求項７】　配列番号６のアミノ酸配列から成るペプチド。

【請求項８】　配列番号６のアミノ酸配列を含むことから成るペプチド。

【請求項９】　配列番号７のアミノ酸配列から成るペプチド。

【請求項１０】　配列番号７のアミノ酸配列を含むことから成るペプチド。

【請求項１１】　配列番号８のアミノ酸配列から成るペプチド。

【請求項１２】　配列番号８のアミノ酸配列を含むことから成るペプチド。

【請求項１３】　配列番号９のアミノ酸配列から成るペプチド。

【請求項１４】　配列番号９のアミノ酸配列を含むことから成るペプチド。

【請求項１５】　配列番号１０のアミノ酸配列から成るペプチド。

【請求項１６】　配列番号１１のアミノ酸配列から成るペプチド。

【請求項１７】　配列番号１２のアミノ酸配列から成るペプチド。

【請求項１８】　配列番号１２のアミノ酸配列を含むことから成るペプチド。

【請求項１９】　配列番号１３のアミノ酸配列から成るペプチド。

【請求項２０】　配列番号１４のアミノ酸配列から成るペプチド。

【請求項２１】　配列番号１４のアミノ酸配列を含むことから成るペプチド。

【請求項２２】　配列番号１５のアミノ酸配列から成るペプチド。

【請求項２３】　配列番号１６のアミノ酸配列から成るペプチド。

【請求項２４】　配列番号１７のアミノ酸配列から成るペプチド。

【請求項２５】　配列番号１７のアミノ酸配列を含むことから成るペプチド。

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【請求項２６】　配列番号１８のアミノ酸配列から成るペプチド。

【請求項２７】　配列番号１９のアミノ酸配列から成るペプチド。

【請求項２８】　配列番号１９のアミノ酸配列を含むことから成るペプチド。

【請求項２９】　配列番号２０のアミノ酸配列から成るペプチド。

【請求項３０】　配列番号２０のアミノ酸配列を含むことから成るペプチド。

【請求項３１】　配列番号２１のアミノ酸配列から成るペプチド。

【請求項３２】　配列番号２１のアミノ酸配列を含むことから成るペプチド。

【請求項３３】　配列番号２２のアミノ酸配列から成るペプチド。

【請求項３４】　配列番号２３のアミノ酸配列から成るペプチド。

【請求項３５】　配列番号２３のアミノ酸配列を含むことから成るペプチド。

【請求項３６】　配列番号２４のアミノ酸配列から成るペプチド。

【請求項３７】　配列番号１のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項３８】　配列番号２のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項３９】　配列番号３のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４０】　配列番号３のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４１】　配列番号４のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４２】　配列番号５のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４３】　配列番号６のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４４】　配列番号６のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４５】　配列番号７のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４６】　配列番号７のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４７】　配列番号８のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４８】　配列番号８のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項４９】　配列番号９のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。

【請求項５０】　配列番号９のアミノ酸配列を含むこと

から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５１】　配列番号１０のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５２】　配列番号１１のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５３】　配列番号１２のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５４】　配列番号１２のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５５】　配列番号１３のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５６】　配列番号１４のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５７】　配列番号１４のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５８】　配列番号１５のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項５９】　配列番号１６のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６０】　配列番号１７のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６１】　配列番号１７のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６２】　配列番号１８のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６３】　配列番号１９のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６４】　配列番号１９のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６５】　配列番号２０のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６６】　配列番号２０のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６７】　配列番号２１のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６８】　配列番号２１のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項６９】　配列番号２２のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項７０】　配列番号２３のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項７１】　配列番号２３のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤。
【請求項７２】　配列番号２４のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】この発明は、スギ花粉アレルゲンに特異的に反応するＴ細胞を活性化するペプチド、及び、そのペプチドを有効成分として含んでなる免疫療法剤に関する。

【０００２】
【従来の技術】ここ数十年来、我国においては、春先になるとスギ花粉症による鼻炎や結膜炎を訴える人の数が増加し続けている。スギ花粉症はアルレギー症の一種であり、その主因はスギ花粉中の抗原性物質、すなわち、スギ花粉症アレルゲンであるといわれている。大気中に飛散したスギ花粉がヒトの体内に侵入すると、スギ花粉アレルゲンに対するイムノグロブリンＥ抗体が産生する。この状態で次にスギ花粉が侵入すると、その花粉中のアレルゲンとこのイムノグロブリンＥ抗体が免疫反応を起し、アレルギー症状を呈することとなる。
【０００３】スギ花粉中に抗原性の相違する少なくとも二種類のアレルゲンの存在することが現在までに知られている。その一つは、ヤスエダ等が『ジャーナル・オブ・アレルギー・アンド・クリニカル・イムノロジー』、第７１巻、第１号、第７７～８６頁（１９８３年）に報告しているアレルゲンであり、今日、これは「Ｃｒｙｊ１」と呼称されている。なお、Ｃｒｙｊ１　はその全長アミノ酸配列が決定され、国際出願されている（ＷＯ９３／０１２１３）。
【０００４】もう一つは、タニアイ等『エフ・イー・ビー・エス・レターズ』、第２３９巻、第２号、第３２９～３３２頁（１９８８年）やサカグチ等『アレルギー』、第４５号、第３０９～３１２頁（１９９０年）に報告されているアレルゲンであり、今日、これは「Ｃｒｙ　ｊ　２」と呼称されている。なお、Ｃｒｙ　ｊ　２はその全長アミノ酸配列が決定され、国際出願されている（ＷＯ　９４／１１５１２）。また、 Komiyama らも別個にＣｒｙｊ２の全長アミノ酸配列を決定しているが（ Biochem. Biophys. Res, Comm., vol.201, No.2, 1021-1028, (1994))、ＷＯ　９４／１１５１２記載のアミノ酸配列とはアミノ酸残基が４か所異なっている。
【０００５】スギ花粉中には、通常、ＣｒｙｊｌとＣｒｙｊ２が約５０：１乃至５：１の割合で存在し、花粉症患者から採取した血清の殆どがＣｒｙｊｌにもＣｒｙｊ２にも反応すると云われている。澤谷らは、『アレルギー』、第４２巻、第６号、第７３８～７４７頁（１９９３年）において、Ｃｒｙｊ２は、皮内反応試験やＲＡＳＴ試験において、Ｃｒｙｊｌと同程度の抗原性を発揮すると報告している。
【０００６】このように、スギ花粉アレルゲンが既に幾つか単離され、その性質、性状もある程度解明されたことから、精製スギ花粉アレルゲンをヒトに投与して減感作することにより、スギ花粉症を治療・予防できる見通しがついてきた。最近ではそのための減感作剤も幾つか考案されており、例えば、特開平１－１５６９２６号公報や特開平３－９３７３０号公報には、Ｎ末端からのアミノ酸配列がＡｓｐ－Ａｓｎ－Ｐｒｏ－Ｉｌｅ－Ａｓｐ－Ｓｅｒ又はＡｌａ－Ｉｌｅ－Ａｓｎ－Ｉｌｅ－Ｐｈｅ

－Ａｓｎで表わされるスギ花粉アレルゲンに糖質を共有結合せしめ、生成した複合体を減感作剤としてヒトに投与する提案が為されている。

【０００７】しかしながら、アレルギー症の診断や減感作療法には、通常、高純度のアレルゲンが大量に必要とされ、スギ花粉中のアレルゲンは僅少であるうえに安定性が低く、スギ花粉症の診断剤や減感作剤をスギ花粉だけで賄おうとすると、多大の困難が伴なう。このようなことから、最近のアレルギー疾患の治療・予防においては、これまでのように、患者にアレルゲン全体を投与するのではなく、アレルゲン中のＴ細胞が特異的に認識する最小領域、すなわち、本質的にＴ細胞エピトープのみからなる低分子のペプチドを投与する免疫療法が注目されている。

【０００８】一般に、アレルゲンは、マクロファージなどの抗原提示細胞に取込まれると、そこで消化され、消化断片が免疫提示細胞表層のＨＬＡ（Human Leucocyte Antigen ）蛋白質に結合し、抗原提示されることとなる。抗原提示される断片は、ＨＬＡ蛋白質に対する親和性などにより、アレルゲンにおける一部の特定領域に限られ、斯かる領域のうち、Ｔ細胞が特異的に認識する領域は、通常、「Ｔ細胞エピトープ」と呼称される。実質的にＴ細胞エプトープのみからなるペプチドを投与する免疫療法には、

【０００９】（ｉ） ペプチドがＢ細胞エピトープを欠いている、すなわち、アレルゲンに特異的なイムノグロブリンＥ抗体が反応しないので、従来の粗製又は精製アレルゲンで頻発していたアナフィラキシーなどの副作用が起こり得ない。

(ii) 少量からスタートし、有効投与量に達するまでの期間が、従来の減感作剤に比較して、大幅に短縮できる。

(iii) 経口免疫寛容を誘導し、アレルゲンに対するアレルギー反応を減弱することができる。などの利点がある。

【００１０】

【発明が解決しようとする課題】本発明者らは、上記Ｔ細胞エピトープを構成する最小単位のアミノ酸配列を見出し、本発明を完成した。この発明の第一の課題は、本質的のスギ花粉アレルゲンのＴ細胞エピトープのみからなるペプチドを提供することにある。この発明の第二の課題は、有効成分として上記ペプチドを含んでなる抗スギ花粉症剤を提供することにある。

【００１１】

【課題を解決するための手段】本発明は、（１） 配列番号１のアミノ酸配列から成るペプチド、（２） 配列番号２のアミノ酸配列から成るペプチド、（３） 配列番号３のアミノ酸配列から成るペプチド、（４） 配列番号３のアミノ酸配列を含むことから成るペプチド、（５） 配列番号４のアミノ酸配列から成るペプチド、

（６） 配列番号５のアミノ酸配列から成るペプチド、（７） 配列番号６のアミノ酸配列から成るペプチド、（８） 配列番号６のアミノ酸配列を含むことから成るペプチド、（９） 配列番号７のアミノ酸配列から成るペプチド、（１０）配列番号７のアミノ酸配列を含むことから成るペプチド、

【００１２】（１１）配列番号８のアミノ酸配列から成るペプチド、（１２）配列番号８のアミノ酸配列を含むことから成るペプチド、（１３）配列番号９のアミノ酸配列から成るペプチド、（１４）配列番号９のアミノ酸配列を含むことから成るペプチド、（１５）配列番号１０のアミノ酸配列から成るペプチド、（１６）配列番号１１のアミノ酸配列から成るペプチド、（１７）配列番号１２のアミノ酸配列から成るペプチド、（１８）配列番号１２のアミノ酸配列を含むことから成るペプチド、（１９）配列番号１３のアミノ酸配列から成るペプチド、（２０）配列番号１４のアミノ酸配列から成るペプチド、

【００１３】（２１）配列番号１４のアミノ酸配列を含むことから成るペプチド、（２２）配列番号１５のアミノ酸配列から成るペプチド、（２３）配列番号１６のアミノ酸配列から成るペプチド、（２４）配列番号１７のアミノ酸配列から成るペプチド、（２５）配列番号１７のアミノ酸配列を含むことから成るペプチド、（２６）配列番号１８のアミノ酸配列から成るペプチド、（２７）配列番号１９のアミノ酸配列から成るペプチド、（２８）配列番号１９のアミノ酸配列を含むことから成るペプチド、（２９）配列番号２０のアミノ酸配列から成るペプチド、（３０）配列番号２０のアミノ酸配列を含むことから成るペプチド、

【００１４】（３１）配列番号２１のアミノ酸配列から成るペプチド、（３２）配列番号２１のアミノ酸配列を含むことから成るペプチド、（３３）配列番号２２のアミノ酸配列から成るペプチド、（３４）配列番号２３のアミノ酸配列から成るペプチド、（３５）配列番号２３のアミノ酸配列を含むことから成るペプチド、（３６）配列番号２４のアミノ酸配列から成るペプチド、（３７）配列番号１のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（３８）配列番号２のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（３９）配列番号３のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（４０）配列番号３のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、

【００１５】（４１）配列番号４のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（４２）配列番号５のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（４３）配列番号６のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（４４）配列番号６のアミノ酸配列を含むことから

成るペプチドを有効成分とする抗スギ花粉症剤、（４５）配列番号７のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、

【００１６】（４６）配列番号７のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（４７）配列番号８のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（４８）配列番号８のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（４９）配列番号９のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（５０）配列番号９のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、

【００１７】（５１）配列番号１０のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（５２）配列番号１１のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（５３）配列番号１２のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（５４）配列番号１２のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（５５）配列番号１３のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、

【００１８】（５６）配列番号１４のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（５７）配列番号１４のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（５８）配列番号１５のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（５９）配列番号１６のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症＊剤、（６０）配列番号１７のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、

【００１９】（６１）配列番号１７のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（６２）配列番号１８のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（６３）配列番号１９のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（６４）配列番号１９のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（６５）配列番号２０のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、

【００２０】（６６）配列番号２０のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（６７）配列番号２１のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（６８）配列番号２１のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（６９）配列番号２２のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（７０）配列番号２３のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、（７１）配列番号２３のアミノ酸配列を含むことから成るペプチドを有効成分とする抗スギ花粉症剤、（７２）配列番号２４のアミノ酸配列から成るペプチドを有効成分とする抗スギ花粉症剤、に関する。

【００２１】以下、本発明を詳しく説明する。本発明における好ましいペプチドの例は表１の通りである。

【００２２】

【表１】

(1) Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser	(ペプチド１)
(2) Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser	(ペプチド２)
(3) Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser	(ペプチド３)
(4) Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly	(ペプチド４)
(5) Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met	(ペプチド５)
(6) Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu	(ペプチド６)
(7) His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln	(ペプチド７)
(8) Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe	(ペプチド８)
(9) Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser	(ペプチド９)
(10) Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp	(ペプチド１０)
(11) Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu	(ペプチド１１)
(12) Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu	(ペプチド１２)
(13) Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met	(ペプチド１３)
(14) Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu	(ペプチド１４)
(15) Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met	(ペプチド１５)
(16) Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly	(ペプチド１６)
(17) Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn	(ペプチド１７)
(18) Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr	(ペプチド１８)
(19) Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn	(ペプチド１９)
(20) Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu	(ペプチド２０)
(21) Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu	(ペプチド２１)

(22)Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn （ペプチド２２）
(23)Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn （ペプチド２３）
(24)Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp （ペプチド２４）

【００２３】なお、上記のペプチド１は、配列表の配列番号１のアミノ酸配列で示されるペプチド、上記のペプチド２は、配列表の配列番号２のアミノ酸配列で示されるペプチド、上記のペプチド３は、配列表の配列番号３のアミノ酸配列で示されるペプチド、上記のペプチド４は、配列表の配列番号４のアミノ酸配列で示されるペプチド、上記のペプチド５は、配列表の配列番号５のアミノ酸配列で示されるペプチド、上記のペプチド６は、配列表の配列番号６のアミノ酸配列で示されるペプチド、上記のペプチド７は、配列表の配列番号７のアミノ酸配列で示されるペプチド、上記のペプチド８は、配列表の配列番号８のアミノ酸配列で示されるペプチド、

【００２４】上記のペプチド９は、配列表の配列番号９のアミノ酸配列で示されるペプチド、上記のペプチド１０は、配列表の配列番号１０のアミノ酸配列で示されるペプチド、上記のペプチド１１は、配列表の配列番号１１のアミノ酸配列で示されるペプチド、上記のペプチド１２は、配列表の配列番号１２のアミノ酸配列で示されるペプチド、上記のペプチド１３は、配列表の配列番号１３のアミノ酸配列で示されるペプチド、上記のペプチド１４は、配列表の配列番号１４のアミノ酸配列で示されるペプチド、上記のペプチド１５は、配列表の配列番号１５のアミノ酸配列で示されるペプチド、

【００２５】上記のペプチド１６は、配列表の配列番号１６のアミノ酸配列で示されるペプチド、上記のペプチド１７は、配列表の配列番号１７のアミノ酸配列で示されるペプチド、上記のペプチド１８は、配列表の配列番号１８のアミノ酸配列で示されるペプチド、上記のペプチド１９は、配列表の配列番号１９のアミノ酸配列で示されるペプチド、上記のペプチド２０は、配列表の配列番号２０のアミノ酸配列で示されるペプチド、上記のペプチド２１は、配列表の配列番号２１のアミノ酸配列で示されるペプチド、上記のペプチド２２は、配列表の配列番号２２のアミノ酸配列で示されるペプチド、上記のペプチド２３は、配列表の配列番号２３のアミノ酸配列で示されるペプチド、上記のペプチド２４は、配列表の配列番号２４のアミノ酸配列で示されるペプチド、をそれぞれ表す。

【００２６】上記（１）乃至（３６）に記載のペプチドは、「固相法」又は「液相法」として知られる斯界において慣用のペプチド合成法により、容易に調製することができる。例えば、社団法人日本生化学会編『新生化学実験講座』、第１巻、「タンパク質ＶＩ」、第３～４４頁、１９９２年、東京化学同人発行などにはペプチド合成の詳細が記載されている。また、該ペプチドは、マルチペプチドシンセサイザー SYMPHONY （プロティンテクノロジー社製）を用い、Fmoc (9-fluorenyl methyloxycarbonyl) 固相合成法にて同装置のプロトコールに従って合成することができる。すなわち、合成する各ペプチドのＣ末端に相当するアミノ酸が導入されている Fmoc-L-アミノ酸 Wang 樹脂を上記ペプチド合成装置の反応容器にセットし、デプロテクション溶液を用いて Fmoc を除く。さらにＣ末端から２番目のアミノ酸に相当するアミノ酸溶液とアクチベーター溶液を反応せしめ、反応後再び Fmoc 基のデプロテクションを行い、同様の操作を繰り返すことにより、目的とするペプチドを合成することができる。

【００２７】本発明のペプチドは化学合成により調製されたものに限定されず、例えば、スギの花粉又は雄花から採取するか、組換えＤＮＡ技術により調製したスギ花粉アレルゲンを適宜分解し、分解物から採取したものであってもよく、例えば、上記（１）乃至（３６）に記載されたペプチドをコードするＤＮＡを調製し、これを自律複製可能なベクターに挿入して組換えＤＮＡとし、これを大腸菌、枯草菌、放線菌、酵母などの適宜宿主に導入して形質転換体とし、その培養物からこの発明のペプチドを採取してもよい。

【００２８】さらに、この発明のペプチドは、斯くして得られるペプチドに糖質やポリエチレングリコールを付加して得られる複合体としての形態、さらには、ペプチドをアセチル化、アミド化及び／又は多官能試験により架橋重合させて得られる誘導体又は重合体としての形態であってもよい。

【００２９】この発明のペプチドは、比較的粗な形態で投与しても所期の治療・予防効果を発揮するが、通常は使用に先立って精製される。精製には、例えば、濾過、濃縮、遠心分離、ゲル濾過クロマトグラフィー、イオン交換クロマトグラフィー、高速液体クロマトグラフィー、アフィニティークロマトグラフィー、ゲル電気泳動、等電点電気泳動などのペプチド乃至蛋白質を精製するための斯界における慣用の方法が用いられ、必要に応じて、これら方法を適宜組合せればよい。そして、最終使用形態に応じて、精製したペプチドを濃縮、凍結乾燥して液状又は固状にすればよい。

【００３０】本発明のペプチドがＴ細胞エピトープとしての活性を有することは、スギ花粉アレルゲンに特異的なＴ細胞の ^{3}Ｈ－チミジンの取込みを計測することにより確認することができる。この計測には、例えば以下の方法を用いることができる。すなわち、フィコール・ハイパック比重遠心法等により花粉症患者の末梢血またはＣｒｙｊ２で免疫したマウス等の実験動物からＣｒｙｊ２に特異的なＴ細胞を含む単核細胞群を分離し、この細

胞群をRPMI 1640 等の培地に浮遊させ、９６ウェルマイクロプレート上に分注する。次に被検物質であるペプチドを加えインキュベートする。このインキュベートの温度・時間は各実験毎に適宜調整することができるが、３７℃、２日間が好適である。その後 ^{3}Ｈ－チミジンを培地に加え、さらに一定時間インキュベーションを続け、単核細胞群における ^{3}Ｈ－チミジンの取り込み量を測定することにより、本発明のペプチドのＴ細胞エピトープとしての活性を算定することができる。なお、本発明では、同時にペプチドを含まない系を設けてこれを陰性対照とし、 ^{3}Ｈ－チミジンの取り込み量が陰性対照の２倍以上に達した系を「陽性」、達しなかった系を「陰性」とした。

【００３１】スギ花粉アレルゲンに特異的なＴ細胞の ^{3}Ｈ－チミジンの取込みの計測は、以下の方法によっても行うことができる。予めマウス等の実験動物をＣｒｙｊ２で免疫し、その後顎下リンパ節等よりリンパ球を採取する。その後、上記と同様の方法により被検体であるペプチドで刺激し、 ^{3}Ｈ－チミジンの取り込み量を測定することにより、本発明のペプチドのＴ細胞エピトープとしての活性を算定することができる。ペプチドの「陽性」及び「陰性」の判定は、上記と同様の基準で行った。

【００３２】本発明のペプチドが花粉症患者に予防効果を有することは、例えば以下の実験により確認することができる。予めマウス等の実験動物に対し本発明のペプチドを投与し、該ペプチドに対する免疫寛容を誘導しておく。一定期間経過後に当該実験動物にＣｒｙｊ２をコレラ毒素等のアジュバントとともに投与し免疫する。さらに、一定期間経過後に当該実験動物より顎下リンパ節細胞を摘出し細胞懸濁液を調製する。

【００３３】また、これとは別の無処理の実験動物より脾臓を抽出し脾臓細胞懸濁液を調製して、これにＸ線を照射し細胞増殖活性を消失させこれを抗原提示細胞含有懸濁液とする。このものを先の顎下リンパ節細胞懸濁液と混合し、これにＣｒｙｊ２を添加して培養を継続し、さらに ^{3}Ｈ－チミジンを添加して、このものの取り込みを測定し、Ｔ細胞の増殖を測定することができる。

【００３４】予め本発明のペプチドで免疫寛容を誘導していない動物では、Ｃｒｙｊ２による免疫化によりそのＴ細胞が抗原提示細胞に結合したＣｒｙｊ２に反応し増殖する。一方、予め本発明のペプチドで免疫寛容を誘導した動物では、その後Ｃｒｙｊ２による免疫を行ってもＴ細胞が抗原提示細胞に結合したＣｒｙｊ２に反応せず増殖しない。その差を測定することにより、本発明のペプチドの花粉症に対する予防効果を確認することができる。

【００３５】さらに、上述の免疫動物の顎下リンパ節細胞懸濁液と抗原提示細胞含有懸濁液の混合液にＣｒｙｊ２を添加して培養を継続した場合に培養液中にインターロイキン４等のサイトカインが分泌されるが、本発明のペプチドを前投与し免疫寛容誘導を行った実験動物と前投与しなかった実験動物とで、このサイトカインの分泌量を比較することによっても、本発明のペプチドの花粉症に対する予防効果を確認することができる。

【００３６】本発明のペプチドが花粉症患者に治療効果を有することは、例えば以下の実験により確認することができる。予めマウス等の実験動物に対し、Ｃｒｙｊ２をコレラ毒素のアジュバンドとともに投与し免疫する。一定期間経過後に当該実験動物にＣｒｙｊ２をコレラ毒素のアジュバンドとともに投与し追加免疫する。さらに、一定期間経過後に当該実験動物より顎下リンパ節細胞を摘出し細胞懸濁液を調製した後、上記と同様の方法によりＴ細胞の増殖を測定する。

【００３７】本発明のペプチドで治療を施していない動物では、Ｃｒｙｊ２による免疫によりそのＴ細胞が抗原提示細胞に結合したＣｒｙｊ２に反応し増殖する。一方、本発明のペプチドで治療した動物では、その後Ｃｒｙｊ２による免疫を行ってもＴ細胞が抗原提示細胞に結合したＣｒｙｊ２に反応せず増殖しない。その差を測定することにより、本発明のペプチドの花粉症に対する治療効果を確認することができる。

【００３８】

【作　用】本発明のペプチドは、スギ花粉アレルゲンに特異的なイムノグロブリンＥ抗体に実質的に反応しないので、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起こすことなく、スギ花粉アレルゲンに特異的なＴ細胞を活性化することができる。有効成分としてかかるペプチドを含んでなる本発明の抗スギ花粉症剤は、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起こすことなくスギ花粉症に対して顕著な治療・予防効果を発揮する。

【００３９】有効成分としてこの発明のペプチドを含んでなる抗スギ花粉症剤は、スギ花粉症に罹患してヒトを含む哺乳類一般に投与すると、アナフィラキシーなどの副作用を実質的に引起こすことなく、スギ花粉症を治療することができる。一方、この発明の抗スギ花粉症例を、スギ花粉が飛散し始める前に健常な個体や潜在的なスギ花粉症の個体に投与するときには、スギ花粉症に対して顕著な予防効果を発揮するとともに、発症時のアレルギー症状の緩解に著効を発揮する。

【００４０】この発明の抗スギ花粉症剤につきさらに詳しく説明すると、この発明の抗スギ花粉症剤は、通常、この発明によるペプチドの１種又は２種以上を0.01乃至１００％(w/w)、望ましくは、0.05乃至５０％(w/v)、さらに望ましくは、0.5乃至5.0％(w/w) 含んでなる。この発明の抗スギ花粉症剤は、当該ペプチド単独の形態はもとより、その以外の生理的に許容される、例えば、血清アルブミン、ゼラチン、マンニトールなどの担体、賦形剤、免疫助成剤、安定剤、さらには、必要に

応じて、ステロイドホルモンやクリモグリク酸ナトリウムなどの抗炎症剤や抗ヒスタミン剤を含む1種又は2種以上の他の薬剤との組成物としての形態を包含する。さらに、この発明の抗スギ花粉症剤は、投薬単位形態の薬剤をも包含し、その投薬単位形態の薬剤とは、この発明のポリペプチドを、例えば、1日当たりの用量又はその整数倍.(4倍まで)又はその約数(1／40まで)に相当する量を含有し、投与に適する物理的に分離した一体の剤形にある薬剤を意味する。このような投薬単位形態の薬剤としては、散剤、細粒剤、顆粒剤、丸剤、錠剤、カプセル剤、トローチ剤、シロップ剤、乳剤、軟鋼剤、硬膏剤、パップ剤、坐剤、点眼剤、点鼻剤、噴霧剤、注射剤などが挙げられる。

【0041】この発明の抗スギ花粉症剤の使用方法について説明すると、この発明の抗スギ花粉症剤は、スギ花粉症の治療・予防を目的に、ヒトを含む哺乳類一般に経皮、経口、点鼻、点眼又は注射投与される。ヒトにおける投与量は、投与の目的や症状に依っても変わるが、通常、対象者の症状や投与後の経過を観察しながら、成人1日当たり0.01乃至1.0g、望ましくは、0.01乃至0.1gを目安に、毎週1回乃至毎月1回の頻度で、約1乃至6カ月間、通常、用量を増やしながら反復投与される。

【0042】本発明のポリペプチドの急性毒性

常法により、生後20日のマウスに後述の製剤例1乃至4の方法により得た免疫治療剤を経口又は腹腔内投与した。その結果、これら免疫療法剤は、いずれの投与経路＊によって200mg/kg以上のLD_{50}であることが判明した。このことは、この発明のペプチドが、ヒトを含むほ乳類に対する免疫療法剤に安全に配合使用し得ることを示している。

【0043】試験例1．スギ花粉症患者より単離したT細胞を用い、本発明のペプチド1乃至ペプチド6、及びペプチド9乃至ペプチド24がスギ花粉抗原T細胞エピトープ活性を有することを確認した。皮膚テストにおいて、スギ花粉アレルゲンに対し陽性を示し、かつ、抗スギ花粉アレルゲン IgE 反応に陽性を示す患者から20mlの末梢血を採取した。遠心分離後、バフィーコートを得て、更にフィコール・パック比重遠心法により、末梢血単核球(Peripheral Blood Mononuelear Cells：PBMC)を採取した。このPBMCを培地(RPMI-1640、5%の熱不活性化ヒトAB型血清を含む。)に、7.5×10^5細胞／mlになるように懸濁した。

【0044】96ウェルの丸底プレートにおいて、1.5×10^5の細胞を、各ウェル200μlの培地中で20ngのペプチドと37℃5%CO_2存在下で48時間培養した。その後、1μCiのトリチウム化チミジンを加え、さらに16時間培養した。細胞に取り込まれたカウントを測定するため、セルハーベスターを用いて細胞をガラス繊維フィルター上に集め、液体シンチレーションカウンターで測定した。この結果を以下の表2に示す。

【0045】

【表2】

ペプチド	T細胞エピトープ活性
ペプチド1	陽　性
ペプチド2	陽　性
ペプチド3	陽　性
ペプチド4	陽　性
ペプチド5	陽　性
ペプチド6	陽　性
ペプチド9	陽　性
ペプチド10	陽　性
ペプチド11	陽　性
ペプチド12	陽　性
ペプチド13	陽　性
ペプチド14	陽　性
ペプチド15	陽　性
ペプチド16	陽　性
ペプチド17	陽　性
ペプチド18	陽　性
ペプチド19	陽　性
ペプチド20	陽　性
ペプチド21	陽　性
ペプチド22	陽　性

15	16
ペプチド２３	陽　性
ペプチド２４	陽　性

以上の結果より、これらのペプチドは、Ｃｒｙｊ２アレルゲンのＴ細胞エピトープを含有していることが示された。

【００４６】試験例２．Ｃｒｙｊ２を文献記載の方法（Allergy, 1990, 45, 309-312）で精製した。精製したＣｒｙｊ２ １μg とコレラ毒素Ｂサブユニット１μg（コレラ毒素0.5％含有）を0.01M リン酸緩衝液（pH 7.4）に溶解させた抗原溶液を、アバチン麻酔下の Balb/c マウス（５～６週齢：チャールズリバージャパン社）に点鼻投与し免疫した。その２週間後、再び同様の方法により同マウスを追加免疫した。その１週間後、マウスの顎下リンパ節細胞を摘出した。これをナイロンメッシュに通し、さらに培地（RPMI 1640 10%子牛胎児血清含有）に懸濁して懸濁液を調製した。

【００４７】また、Ｃｒｙｊ２で免疫化していないマウスより脾臓細胞を摘出し、上記と同様の方法でリンパ節細胞懸濁液を調製した。この懸濁液に3000 RadのＸ線を照射して細胞の増殖活性を消失させ、抗原提示細胞懸濁液として用いた。平底９６ウェルプレート（コーニング社）に、１ウェル当たりリンパ節細胞３×１０^{5}、抗原提示細胞６×１０^{5}となるように分注し、ペプチド７又はペプチド８の存在下（0.5μg/ml）、あるいはこれらペプチドの非存在下で、３７℃、５％CO_2 の条件下３日間培養した。

【００４８】最後の１６時間は、^{3}H-Thymidine 存在下で培養し、この間に細胞核内ＤＮＡに取り込まれた ^{3}H-Thymidine 量を、ガラスフィルターに吸着したＤＮＡの放射線量を液体シンチレーション法により測定することにより算定した。ペプチド存在下での ^{3}H-Thymidine 取り込み量を、ペプチド非存在下での取り込み量で割った値を反応倍率として、これを細胞増殖活性の指標とした。

【００４９】リンパ節細胞は、ペプチド７に対しては３倍程度、ペプチド８に対しては５倍程度増殖率が増大した。従って、これらのペプチドは、Ｃｒｙｊ２アレルゲンのＴ細胞エピトープを含有していることが示された。

【００５０】試験例３．ペプチド７又は８について、Balb/c マウスに対して免疫寛容を誘導した。すなわち、リン酸緩衝液（0.01M (pH 7.4)）に溶解させた各ペプチド溶液について、マウス尾静脈に一匹当たり２０μg のペプチド量となるように静脈投与を行った。または、同ペプチド溶液を、１匹１回当たり１mgのペプチド量となるように経口投与を行い、この経口投与を２週間に４回繰り返した。その後、当該マウスについて、試験例２と同様の方法で、Ｃｒｙｊ２による免疫を行った。

【００５１】試験例２と同様の方法で該マウスより顎下リンパ節細胞を摘出して顎下リンパ節細胞懸濁液とし、また、ペプチドによる寛容化とＣｒｙｊ２による免疫誘導を行っていない別個のマウスより脾臓を摘出してＸ線により増殖活性を消失させ抗原提示細胞懸濁液として、これらをＣｒｙｊ２の存在（１μg/ml）下で共培養して、試験例２と同様の方法で ^{3}H-Thymidine 取り込み量を測定し細胞増殖活性を算定した。

【００５２】また、リンパ節細胞及び抗原提示細胞の懸濁液を調製培地により調製した。１ウェル当たりリンパ節細胞1.５×１０^{5}、抗原提示細胞３×１０^{5}となるように、２４ウェルプレート（コーニング）に分注し、これらの細胞をＣｒｙｊ２（１μg/ml）と共に３７℃、５％CO_2 の条件下で３日間培養した。培養終了後、培養上清液を採取し、測定に用いるまで２０℃で凍結保存した。培養液中に含まれるインターロイキン４の量を市販の測定キット（Endogen 社）にて測定した。

【００５３】（１）ペプチド７の静脈投与による免疫寛容の誘導

マウス尾静脈にペプチド７の溶液を投与した。対照群のマウスには、リン酸緩衝液（0.01M (pH 7.4)）のみを静脈投与した。その後、上記の方法に従って、両群のマウスをＣｒｙｊ２で経鼻的に免疫した。その後、当該マウスより摘出した顎下リンパ節細胞及び他のマウスより摘出した抗原提示細胞をＣｒｙｊ２と共に培養すると、あらかじめペプチド７を投与したマウスからのリンパ節細胞の増殖活性は、対照群に比較して29.5%低下していた。これによりペプチド７には、スギアレルゲンに対する免疫応答を抑制する活性があることが明らかとなった。

【００５４】（２）ペプチド７の経口投与による免疫寛容の誘導

マウスにＴ細胞ペプチド７の溶液を２週間の間に４回、上記の方法に従い経口投与した。対照群のマウスには、リン酸緩衝液（0.01M (pH 7.4)）のみを経口投与した。その後、両群のマウスをＣｒｙｊ２で経鼻的に免疫した。その後、当該マウスより摘出した顎下リンパ節細胞及び他のマウスより摘出した抗原提示細胞をＣｒｙｊ２と共に３日間培養し、その培養上清中のサイトカイン量を測定した。その結果、あらかじめペプチド７を投与したマウスからのリンパ節細胞から産生されるインターロイキン４の量は、対照群に比較して49.8%低下していた。これによりペプチド７を経口的に投与することにより、スギアレルゲンに対する免疫応答を抑制することが示された。

【００５５】（３）ペプチド８の静脈投与による免疫寛容の誘導

マウス尾静脈にペプチド８の溶液を投与した。対照群のマウスには、リン酸緩衝液（0.01M (pH 7.4)）のみを静

脈投与した。その後、上記の方法に従って、両群のマウスをＣｒｙｊ２で経鼻的に免疫した。その後、当該マウスより摘出した顎下リンパ節細胞及び他のマウスより摘出した抗原提示細胞をＣｒｙｊ２と共に培養すると、あらかじめペプチド８を投与したマウスからのリンパ節細胞の増殖活性は、対照群に比較して30.9%低下していた。これによりペプチド８には、スギアレルゲンに対する免疫応答を抑制する活性があることが明らかとなった。

【００５６】（４）ペプチド８の経口投与による免疫寛容の誘導

マウスにＴ細胞ペプチド８の溶液を2週間の間に４回、上記の方法に従い経口投与した。対照群のマウスには、リン酸緩衝液（0.01M (pH 7.4)）のみを経口投与した。その後、両群のマウスをＣｒｙｊ２で経鼻的に免疫した。その後、当該マウスより摘出した顎下リンパ節細胞及び他のマウスより摘出した抗原提示細胞をＣｒｙｊ２と共に培養すると、あらかじめペプチド８を投与したマウスからのリンパ節細胞の増殖活性は、対照群に比較して73.1%低下していた。これによりペプチド８には、スギアレルゲンに対する免疫応答を抑制する活性があることが明らかとなった。

【００５７】試験例４

ペプチド８について、Balb/cマウスに対して、治療を施した。すなわち、精製したＣｒｙｊ２　１μｇとコレラ毒素Ｂサブユニット１μｇ（コレラ毒素０．５%含有）を0.01Mリン酸緩衝液（ｐＨ７．４）に溶解させた抗原溶液を、アバチン麻酔下の２群のBalb/cマウス（５～６週齢：チャールズリバージャパン社）に点鼻投与し免疫した。一週間後より、実験群のマウスに対して、0.01Mリン酸緩衝液（ｐＨ７．４）に溶解させたペプチド８の溶液を、一匹について一回あたり200 μｇのペプチド量となるように経口投与し、この経口投与を２週間の間に４回繰り返した。対照群のマウスには、0.01Mリン酸緩衝液（ｐＨ７．４）のみを同様に投与した。４回目の経口投与から４日後に、両群のマウスに再度Ｃｒｙｊ２で経鼻的に免疫した。一週間後、試験例３と同様の方法により当該マウスより摘出した顎下リンパ節細胞と他のマウスより摘出した抗原提示細胞とをＣｒｙｊ２と共に培養すると、実験群マウス由来のリンパ節細胞の増殖は、対照群に比較して46.0%低下していた。この結果より、ペプチド８は、スキアレルゲンで免疫された後のマウスに投与した場合にも、スキアレルゲンに対する免疫応答を抑制する活性を有することが明らかとなった。

【００５８】以上のように、本発明のペプチドは、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起こすことなく、スギ花粉アレルゲンに特異的なＴ細胞を活性化することができる。有効成分として斯かるペプチドを含んでなる本発明の抗スギ花粉症剤は、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起こすことなくスギ花粉症に対して顕著な治療・予防効果を発揮する。

【００５９】有効成分としてこの発明のペプチドを含んでなる抗スギ花粉症剤は、スギ花粉症に罹患してヒトを含む哺乳類一般に投与すると、アナフィラキシーなどの副作用を実質的に引起こすことなく、スギ花粉症を治療することができる。一方、この発明の抗スギ花粉症剤を、スギ花粉が飛散し始める前に健常な個体や潜在的なスギ花粉症の個体に投与するときには、スギ花粉症に対して顕著な予防効果を発揮するとともに、発症時のアレルギー症状の緩解に著効を発揮する。

【００６０】

【発明の実施の形態】以下、実施例、製剤例により本発明をさらに詳細に説明するが、本発明はこれらによりその技術的範囲が限定されるものではない。

実施例１

ペプチド１：

Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser

樹脂に固定したアミノ酸誘導体に１個ずつアミノ酸をカルボキシル末端側から結合させていく方法（固相合成法）でペプチドを化学合成した。各サイクルで使用するアミノ酸はαアミノ基及び残基部分の反応基が保護基でブロックされた特殊なアミノ酸誘導体を用いた。ここで、それぞれのαアミノ基が Fmoc (9-fluorenyl methyloxycarbonyl) によりブロックされているアミノ酸を用いた（Fmoc法）。また、ペプチド合成は樹脂に結合したアミノ酸のαアミノ基の Fmoc を脱保護し、次にカルボキシル基が活性化したアミノ酸誘導体を結合させるという反応を順次繰り返して行った。

【００６１】実験に用いる各ペプチドは、マルチペプチドシンセサイザー SYMPHONY (Protein Technologies, Inc.)を用い上記の Fmoc 固相合成法にて同装置のプロトコールに従って合成した。すなわち、合成するペプチドのＣ末端残基に相当するアミノ酸（Ser）が導入されている Fmoc-Ser(tBu)-Wang-樹脂（0.52mmol/g）の２５μmol 相当を上記ペプチド合成装置の反応容器にセットし、デブロテクション溶液（20% piperidine / Dimethyl formamide (DMF)）1.25mlを５分間２回反応させ、樹脂に結合しているアミノ酸の Fmoc 基を除いた。DMF 液1.25mlで３０秒間６回洗浄後、Ｃ末側から２番目のアミノ酸に相当する２００mMの Fmoc-Ala/DMF 溶液1.25mlと２００mMのアクチベータ溶液(200mM O-Benzotriazole-N,N,',N',-Tetramethyl-Uronium-Hexafluoro phosphate／400mM N-methylmorpholine／DMF) 1.25mlを加え（それぞれ理論等量の10倍：250μmol 相当）、２０分間室温で反応させた。ここで生成した Fmoc-Ala-Ser(tBu)-Wang-樹脂をDMF 1.25mlにて３０秒間６回洗浄後、再び Fmoc 基のデブロテクションを用い、DMF 1.25mlにて３０秒間６回洗浄後、Fmoc-Pro 溶液とアクチベーター

溶液を加え反応させた。同様の操作を繰り返すことにより、目的とするペプチド（Fmoc-Lys(Boc)-Val-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang- 樹脂）を合成した。

＊ Fmoc-Ala, Fmoc-Pro, Fmoc-Asn(Trt), Fmoc-Gln(Trt), Fmoc-Tyr(tBu), Fmoc-Ile, Fmoc-Gly, Fmoc-Asp(OtBu), Fmoc-Val, Fmoc-Lys(Boc),

ペプチド合成装置 SYMPHONY を用い、装置内でクリベージ反応を行った。

【００６３】まず、上記ように合成し得られた保護ペプチド樹脂（Fmoc-Lys(Boc)-Val-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang- 樹脂）に、デブロテクション液1.25mlを５分間２回反応させてＮ末端Fmoc基を脱保護した。次に1.25mlのDMF にて３０秒間６回洗浄後、CH_2Cl_2 にて同様に洗浄し、 N_2を吹き付け１０分間乾燥後、クリベージ溶液（Trifluoroacetic acid：Phenol：水：Tioanisole：Ethanedithiol ＝82.5：５：５：５：2.5）を2.5ml加え室温で２時間反応させ（D.S.King, Int.J.Peptide Protein Reg., 36, 255(1990)）、樹脂からのペプチドの切断およびアミノ酸側鎖保護基の除去を行い、ペプチド（Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser）を得た。

【００６４】反応終了後、このペプチド溶液をフィルターを用いて濾過し、樹脂と濾液に分けた。さらに樹脂を洗浄した液2.5mlと合わせ遠心管に回収した。回収したペプチド溶液を装置から取り出し、５mlの冷エーテルを加え、ペプチドを沈澱させた。しばらく冷却後これを遠心して（3000rpm 10分間）沈澱物を集め、再び冷エーテルを加えて分散させては回収することを５～６回繰り返してペプチドを洗浄した。

【００６５】得られたペプチドを乾燥させ、粗ペプチドを得た（50.5mg）。粗ペプチドは0.１％ TFAを含む１０％アセトニトリル水溶液に溶解後、ODS カラム(TSKgel ODS-120T, 21.5mm×30cm：東ソー（株）製）に供与し、0.１％ TFAを含む２１％アセトニトリルにて展開し（流速９ml／分、検出波長 220nm）、３１～３５分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（15.9mg）。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００６６】実施例２

ペプチド２：

Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser

実施例１と同様の操作でペプチド（Fmoc-Val-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-樹脂）を合成し、クリベージ反応を行いペプチド（Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser）を得、このペプチド溶液を遠心管に回収した。その後、ペプチドを沈澱させ、粗ペプチドを得た（55.5mg）。

【００６７】粗ペプチドは0.１％ TFAを含む１０％アセトニトリル水溶液に溶解後、ODS カラム(TSKgel ODS-120T, 21.5mm×30cm：東ソー（株）製）に供与し、0.１％ TFAを含む２２％アセトニトリルにて展開し（流速９ml／分、検出波長 220nm）、２６～２９分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（7.1mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００６８】実施例３

ペプチド３：

Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser

実施例１と同様の操作でペプチド（Fmoc-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-樹脂）を合成し、クリベージ反応を行いペプチド（Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser）を得、このペプチド溶液を遠心管に回収した。その後、ペプチドを沈澱させ、粗ペプチドを得た（47.9mg）。

【００６９】粗ペプチドは0.１％ TFAを含む１０％アセトニトリル水溶液に溶解後、ODS カラム(TSKgel ODS-120T, 21.5mm×30cm：東ソー（株）製）に供与し、0.１％ TFAを含む２１％アセトニトリルにて展開し（流速９ml／分、検出波長 220nm）、２５～２８分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（13.8mg）。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００７０】実施例４

ペプチド４：

Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly

実施例１と同様の操作でペプチド（Fmoc-Trp-Leu-Gln-(Trt)-Phe-Ala-Lys(Boe)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Wang- 樹脂）を合成した。ただし、C末端アミノ酸樹脂には Fmoc-Gly-Wang- 樹脂（0.50mol

ベージ/g）を２５μmol 相当用いた。合成に使用したアミノ酸は以下のとおりである。

Fmoc-Met, Fmoc-Leu, Fmoc-Thr(tBu),
Fmoc-Phe, Fmoc-Gly, Fmoc-Lys(Boc),
Fmoc-Ala, Fmoc-Gln(Trt), Fmoc-Trp,

実施例１と同様の操作でクリベージ反応を行いペプチド(Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly ）を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た（63.3mg）。

【００７２】粗ペプチドは0.1％ TFAを含む２０％アセトニトリル水溶液に溶解後、ODS カラム(TSKgel ODS-120T, 21.5mm×30cm：東ソー（株）製）に供与し、0.1％ TFAを含む３８％アセトニトリルにて展開し（流速９ml／分、検出波長 220nm）、２５～３１分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（2.0mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００７３】実施例５

ペプチド５：

Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met

実施例１と同様の操作でペプチド（Fmoc-Trp-Leu-Gln-(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Wang- 樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Met-Wang- 樹脂（0.75mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は実施例４と同じである。実施例１と同様の操作でクリベージ反応を行いペプチド(Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met- ）を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た（29mg）。

【００７４】粗ペプチドは0.1％ TFAを含む２０％アセトニトリル水溶液に溶解後、ODS カラム(TSKgel ODS-120T, 21.5mm×30cm：東ソー（株）製）に供与し、0.1％ TFAを含む３６％アセトニトリルにて展開し（流速９ml／分、検出波長 220nm）、３２～３４分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（1.1mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００７５】実施例６

ペプチド６：

Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu

実施例１と同様の操作でペプチド（Fmoc-Trp-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang-樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Leu-Wang- 樹脂（0.69mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は実施例４と同じである。

【００７６】実施例１と同様の操作でクリベージ反応を行いペプチド(Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu ）を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た（35.6mg）。粗ペプチドは0.1％ TFAを含む２０％アセトニトリル水溶液に溶解後、ODS カラム(TSKgel ODS-120T, 21.5mm×30cm：東ソー（株）製）に供与し、0.1％ TFAを含む３８％アセトニトリルにて展開し、２６～３０分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（6.3mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００７７】実施例７

ペプチド７：

His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln

実施例１記載の Fmoc 法により、Milligen / Biosearch 社製 9050 ペプチド合成機を用い、粗ペプチド４００mgを得た。粗ペプチドは0.1％ TFA水溶液に溶解後、μBONDASPHERE 5μ C18C120 Aカラム（19×150mm）に供与し、0.1％ TFAを含む９０％アセトニトリル溶液にて展開し（流速５ml／分、検出波長214nm)、２８～２９分に溶出された画分をエバポレート後、凍結乾燥を行い目的とするペプチドを得た（36mg）。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００７８】実施例８

ペプチド８：

Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe

実施例１記載の Fmoc 法により、Milligen / Biosearch 社製 9050 ペプチド合成機を用い、粗ペプチド５５０mgを得た。粗ペプチドは0.1％ TFA水溶液に溶解後、μBONDASPHERE 5μ C18C120 Aカラム（19×150mm）に供与し、0.1％ TFAを含む９０％アセトニトリル溶液にて展開し（流速５ml／分、検出波長214nm)、２６～２７分に溶出された画分をエバポレート後、凍結乾燥を行い目的とするペプチドを得た（60mg）。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行っ

たところ、上記に示されるアミノ酸配列が確認された。

【００７９】実施例９

ペプチド９：

Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser

樹脂に固定したアミノ酸誘導体に１個ずつアミノ酸をカルボキシル末端側から結合させていく方法（固相合成法）でペプチドを化学合成した。各サイクルで使用するアミノ酸はαアミノ基及び残基部分の反応基が保護基でブロックされた特殊なアミノ酸誘導体を用いた。ここで、それぞれのαアミノ基が Fmoc (9-fluorenyl methyloxycarbonyl) によりブロックされているアミノ酸を用いた（Fmoc法）。また、ペプチド合成は樹脂に結合したアミノ酸のαアミノ基の Fmoc を脱保護し、次にカルボキシル基が活性化したアミノ酸誘導体を結合させるという反応を順次繰り返して行った。

【００８０】実験に用いる各ペプチドは、マルチペプチドシンセサイザー SYMPHONY (Protein Technologies, Inc.)を用い上記の Fmoc 固相合成法にて同装置のプロトコールに従って合成した。すなわち、合成するペプチドのC末端残基に相当するアミノ酸（Ser）が導入されている Fmoc-Ser(tBu)-Wang-樹脂（0.52mmol/g）の２５μ＊mol 相当を上記ペプチド合成装置の反応容器にセットし、デブロテクション溶液（20% piperidine / Dimethyl formamide (DMF)）1.25mlを５分間２回反応させ、樹脂に結合しているアミノ酸の Fmoc 基を除いた。DMF 液1.25mlで３０秒間６回洗浄後、C末側から２番目のアミノ酸に相当する２００mMの Fmoc-Ala/DMF 溶液1.25mlと２００mMのアクチベータ溶液(200mM O-Benzotriazole-N,N,',N',-Tetramethyl-Uronium-Hexafluoro phosphate／400mM N-methylmorpholine／DMF ）1.25mlを加え（それぞれ理論等量の10倍：250μmol 相当）、２０分間室温で反応させた。ここで生成した Fmoc-Ala-Ser(tBu)-Wang-樹脂をDMF 1.25mlにて３０秒間６回洗浄後、再び Fmoc 基のデブロテクションを用い、DMF 1.25mlにて３０秒間６回洗浄後、Fmoc-Pro 溶液とアクチベーター溶液を加え反応させた。同様の操作を繰り返すことにより、目的とするペプチド (Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-樹脂）を合成した。

【００８１】ここで合成に使用したアミノ酸は以下のとおりである（日清紡（株）製）。（　）内は残基部分の反応基を保護する保護基を表す。

＊　Fmoc-Ala,　Fmoc-Pro,
Fmoc-Asn(Trt),　Fmoc-Gln(Trt),　Fmoc-Tyr(tBu),
Fmoc-Ile,　Fmoc-Gly,

ペプチド合成装置 SYMPHONY を用い、装置内でクリベージ反応を行った。

【００８２】まず、上記ように合成し得られた保護ペプチド樹脂（Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)- Wang- 樹脂）に、デブロテクション液1.25mlを５分間２回反応させてN末端Fmoc基を脱保護した。次に1.25mlのDMF にて３０秒間６回洗浄後、CH_2Cl_2 にて同様に洗浄し、 N_2 を吹き付け１０分間乾燥後、クリベージ溶液（Trifluoroacetic acid: Phenol：水：Tioanisole：Ethanedithiol ＝82.5：５：５：５：2.5）を2.5ml加え室温で２時間反応させ（D.S.King, Int.J.Peptide Protein Req., 36, 255(1990))、樹脂からのペプチドの切断およびアミノ酸側鎖保護基の除去を行い、ペプチド（Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser）を得た。

【００８３】反応終了後、このペプチド溶液をフィルターを用いて濾過し、樹脂と濾液に分けた。さらに樹脂を洗浄した液2.5mlと合わせ遠心管に回収した。回収したペプチド溶液を装置から取り出し、５mlの冷エーテルを加え、ペプチドを沈澱させた。しばらく冷却後これを遠心して（3000rpm 10分間）沈澱物を集め、再び冷エーテルを加えて分散させては回収することを５～６回繰り返してペプチドを洗浄した。

※【００８４】得られたペプチドを乾燥させ、粗ペプチドを得た。得られた粗ペプチドのうち11mgを２mlの0.1％TFAを含む１０％アセトニトリル水溶液に溶解後、３回に分けてODS カラム(TSKgel ODS-120T, 7.8mm×30cm：東ソー（株）製）に供与し、0.1％ TFAを含む２１％アセトニトリルにて展開し（流速２ml／分、検出波長 220 nm）、９．２～１１分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（５mg）。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株) 製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００８５】実施例１０

ペプチド１０：

Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp

実施例９と同様の操作でペプチド（Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp-Wang-樹脂）を合成した。ただし、C末端アミノ酸樹脂には Fmoc-Trp-Wang- 樹脂（0.66mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は以下のとおりである。

【００８６】

※　Fmoc-Ala,　Fmoc-Pro,　Fmoc-Asn(Trt),
Fmoc-Gln(Trt),　Fmoc-Tyr(tBu),　Fmoc-Ile,
Fmoc-Gly,　Fmoc-Ser(tBu)

実施例９と同様の操作でクリベージ反応を行いペプチド(Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp)を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【００８７】得られた粗ペプチドのうち９mgを４mlの0.１% TFAを含む１０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30 cm：東ソー（株）製）に供与し、0.１% TFAを含む２３%アセトニトリルにて展開し、３２～３８分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（2.5mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に＊示されるアミノ酸配列が確認された。

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Phe, Fmoc-Gly, Fmoc-Lys(Boc), Fmoc-Ala, Fmoc-Gln(Trt), Fmoc-Trp, Fmoc-Ile,

実施例９と同様の操作でクリベージ反応を行いペプチド(Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu）を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【００９０】得られた粗ペプチドのうち７mgを４mlの0.１% TFAを含む２０%アセトニトリル水溶液に溶解後、３回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30 cm：東ソー（株）製）に供与し、0.１% TFAを含む３７%アセトニトリルにて展開し（流速２ml／分、検出波長 220nm)、１７～２０分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（0.7 mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用い※てアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Phe, Fmoc-Gly, Fmoc-Lys(Boc), Fmoc-Ala, Fmoc-Gln(Trt),

得られた粗ペプチドのうち 9.6mgを２mlの0.１% TFAを含む２０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む３２%アセトニトリルにて展開し（流速２ml／分、検出波長 220nm)、１１～１６分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（６.4mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００９３】実施例１３

ペプチド１３

Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met

実施例９と同様の操作でペプチド（Fmoc-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Wang-樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Met-Wang- 樹脂（0.75mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は実施例１２と同じである。実施例９と同様の操作でクリベージ反応を行いペプチド(Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met）を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【００９４】得られた粗ペプチドのうち８mgを２mlの0.１% TFAを含む２０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30 cm：東ソー（株）製）に供与し、0.１% TFAを含む３０%アセトニトリルにて展開し、２５～３２分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（1.1mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００９５】実施例１４

【００８８】実施例１１

ペプチド１１：

Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu

実施例９と同様の操作でペプチド（Fmoc-Ile-Trp-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang-樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Leu-Wang- 樹脂（0.69mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は以下のとおりである。

【００８９】

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Phe, Fmoc-Gly, Fmoc-Lys(Boc), Fmoc-Ala, Fmoc-Gln(Trt), Fmoc-Trp, Fmoc-Ile,

※てアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００９１】実施例１２

ペプチド１２：

Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu

実施例９と同様の操作でペプチド（Fmoc-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang- 樹脂）を合成し、クリベージ反応を行いペプチド（Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu）を得、このペプチド溶液を遠心管に回収した。その後、ペプチドを沈澱させ、粗ペプチドを得た。合成に使用したアミノ酸は以下のとおりである。

【００９２】

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Phe, Fmoc-Gly, Fmoc-Lys(Boc), Fmoc-Ala,

ペプチド１４：

Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu

実施例９と同様の操作でペプチド（Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Leu-Wang- 樹脂（0.69mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は実施例１２と同じである。実施例９と同様の操作でクリベージ反応を行いペプチド(Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu）を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【００９６】得られた粗ペプチドのうち 2.5mgを１mlの0.１% TFAを含む２０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む３０%アセトニトリルにて展開し、１０～１２分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（0.6mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株）製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００９７】実施例１５

ペプチド１５：

Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met

実施例９と同様の操作でペプチド（Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Wang樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Met-Wang- 樹脂（0.75mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は実施例１２と同じである。実施例９と同様の操作でクリベージ反応を行いペプチド(Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met）を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【００９８】得られた粗ペプチドのうち７mgを４mlの0.１% TFAを含む２０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む３０%アセトニトリルにて展開し、１５～２０分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（1.9mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株）製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【００９９】実施例１６

ペプチド１６：

Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly

実施例９と同様の操作でペプチド（Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Wang樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Gly-Wang- 樹脂（0.50mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は以下のとおりである。

【０１００】

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Phe,
Fmoc-Gly, Fmoc-Lys(Boc), Fmoc-Ala,
Fmoc-Gln(Trt), Fmoc-Met

実施例９と同様の操作でクリベージ反応を行いペプチド(Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly)を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【０１０１】得られた粗ペプチドのうち13mgを６mlの0.１% TFAを含む２０%アセトニトリル水溶液に溶解後、３回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む２９%アセトニトリルにて展開し、１７～２０分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（0.9mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株）製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１０２】実施例１７

ペプチド１７：

Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn

実施例９と同様の操作でペプチド（Fmoc-Ile-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Wang- 樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Asn(Trt)-Wang-樹脂（0.60mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は以下のとおりである。

【０１０３】

Fmoc-Leu, Fmoc-Asn(Trt), Fmoc-Ile,
Fmoc-Phe, Fmoc-Lys(Boc), Fmoc-His(Trt),
Fmoc-Ala, Fmoc-Gln(Trt), Fmoc-Ser(tBu),

実施例９と同様の操作でクリベージ反応を行いペプチド(Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn)を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【０１０４】得られた粗ペプチドのうち 3.8mgを４mlの0.１% TFAを含む１０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む１８%アセトニトリルにて展開し（流速２ml／分、検出波長 220nm)、１２～１５分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（1.9mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株）製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１０５】実施例１８

ペプチド１８：

Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr
実施例９と同様の操作でペプチド（Fmoc-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Wang-樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Thr(tBu)-Wang-樹脂（0.50mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は以下のとおりである。

【０１０６】

Fmoc-Leu, Fmoc-Asn(Trt), Fmoc-Phe,
Fmoc-Lys(Boc), Fmoc-His(Trt), Fmoc-Ala,
Fmoc-Gln(Trt), Fmoc-Ser(tBu),

実施例９と同様の操作でクリベージ反応を行いペプチド(Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr)を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【０１０７】得られた粗ペプチドのうち５mgを４mlの0.１% TFAを含む１０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む１５%アセトニトリルにて展開し、２２～３０分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（3.5mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１０８】実施例１９

ペプチド１９：

Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn

実施例９と同様の操作でペプチド（Fmoc-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Wang- 樹脂）を合成し、クリベージ反応を行いペプチド(Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn）を得、このペプチド溶液を遠心管に回収した。その後、ペプチドを沈澱させ、粗ペプチドを得た。合成に使用したアミノ酸は実施例１８と同じである。

【０１０９】得られた粗ペプチドのうち６mgを４mlの0.１% TFAを含む１０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む１５%アセトニトリルにて展開し、２０～２８分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（3.8mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１１０】実施例２０

ペプチド２０：

Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu

実施例９と同様の操作でペプチド（Fmoc-Leu-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Wang-樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Leu-Wang- 樹脂（0.69mmol/g）を２５μmol相当用いた。合成に使用したアミノ酸は以下のとおりである。

【０１１１】

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Asn(Trt),
Fmoc-Gly, Fmoc-Lys(Boc), Fmoc-Cys(Trt),
Fmoc-Ala, Fmoc-Ser(tBu), Fmoc-Ile

実施例９と同様の操作でクリベージ反応を行いペプチド(Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu)を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【０１１２】得られた粗ペプチドのうち 10mg を４mlの0.１% TFAを含む１０%アセトニトリル水溶液に溶解後、３回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む２３%アセトニトリルにて展開し（流速２ml／分、検出波長 220nm）、１８～２２分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（0.9mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１１３】実施例２１

ペプチド２１：

Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu

実施例９と同様の操作でペプチド（Fmoc-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Wang- 樹脂）を合成し、クリベージ反応を行いペプチド（Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu ）を得、このペプチド溶液を遠心管に回収した。その後、ペプチドを沈澱させ、粗ペプチドを得た。合成に使用したアミノ酸は実施例２０と同じである。

【０１１４】得られた粗ペプチドのうち 6.6mgを２mlの0.１% TFAを含む１０%アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.１% TFAを含む１９%アセトニトリルにて展開し（流速２ml／分、検出波長 220nm）、１７～２２分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（1.5mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１１５】実施例２２

ペプチド２２：

Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn

実施例１と同様の操作でペプチド（Fmoc-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Wang- 樹脂）を合成した。ただ

し、Ｃ末端アミノ酸樹脂には Fmoc-Asn(Trt)-Wang-樹脂（0.60mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は実施例２０と同じである。実施例９と同様の操作でクリベージ反応を行いペプチド(Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu) を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【０１１６】得られた粗ペプチドのうち 6.9mgを１mlの0.1％ TFAを含む１０％アセトニトリル水溶液に溶解後、２回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.1％ TFAを含む２２％アセトニトリルにて展開し、９～１２分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（1.6mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１１７】実施例２３

ペプチド２３：

Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn

実施例９と同様の操作でペプチド（Fmoc-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Wang-樹脂）を合成し、クリベージ反応を行いペプチド（Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn ）を得、このペプチド溶液を遠心管に回収した。その後、ペプチドを沈澱させ、粗ペプチドを得た。合成に使用したアミノ酸は以下のとおりである。

【０１１８】

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Gly, Fmoc-Cys(Trt), Fmoc-Ala, Fmoc-Ser(tBu), Fmoc-Ile

得られた粗ペプチドのうち６mgを１mlの0.1％ TFAを含む２０％アセトニトリル水溶液に溶解後、３回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.1％ TFAを含む１９％アセトニトリルにて展開し（流速２ml／分、検出波長 220nm）、１５～１７分に溶出された画分を分取し、濃縮後、凍結乾燥を行い目的とするペプチドを得た（０.9 mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１１９】実施例２４

ペプチド２４：

Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp

実施例９と同様の操作でペプチド（Fmoc-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Asp(OtBu)-Wang-樹脂）を合成した。ただし、Ｃ末端アミノ酸樹脂には Fmoc-Asp(OtBu)-Wang- 樹脂（0.42mmol/g）を２５μmol 相当用いた。合成に使用したアミノ酸は以下のとおりである。

【０１２０】

Fmoc-Leu, Fmoc-Thr(tBu), Fmoc-Gly, Fmoc-Cys(Trt), Fmoc-Ala, Fmoc-Ser(tBu), Fmoc-Ile Fmoc-Asn(Trt)

実施例９と同様の操作でクリベージ反応を行いペプチド(Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp)を得、このペプチド溶液を遠心管に回収し、その後、ペプチドを沈澱させ、粗ペプチドを得た。

【０１２１】得られた粗ペプチドのうち 7.5mgを１mlの0.1％ TFAを含む１０％アセトニトリル水溶液に溶解後、３回に分けてODS カラム(TSKgel ODS-120T, 7.8mm ×30cm：東ソー（株）製）に供与し、0.1％ TFAを含む１８％アセトニトリルにて展開し、１７～１９分に溶出された画分を濃縮後、凍結乾燥を行い目的とするペプチドを得た（0.6mg)。この合成したペプチド 50 pmolについて、アミノ酸配列分析装置 PPSQ-10型(島津製作所(株)製）を用いてアミノ酸配列分析を行ったところ、上記に示されるアミノ酸配列が確認された。

【０１２２】製剤例１.

液剤

実施例１乃至２４記載の方法により得た２４種類のペプチドのいずれかを最終濃度0.1 g/mlになるように安定剤として１％(w/v) 精製ゼラチンを含む蒸留水に溶解し、常法により滅菌濾過して２４種類の液剤を得た。

【０１２３】本発明のペプチドに対する感受性は個体毎に変わるのが通例であるから、本品は個々の個体に最も適した組成になるよう、２４種類の液剤を適宜配合して使用する。本品は安定性に優れているので、スギ花粉症を治療・予防するための点眼剤、点鼻剤、口腔内噴霧剤用の液剤として有用である。

【０１２４】製剤例２.

注射剤

安定剤として１％(w/v) ヒト血清アルブミンを含む生理食塩水に実施例１乃至２４記載の方法により得た２４種類のペプチドをそれぞれ最終濃度0.01、0.1 又は１mg/mlになるように溶解し、滅菌濾過した後、滅菌バイアル瓶に２mlずつ分注し、凍結乾燥し、密栓した。

【０１２５】本品は投与に先立ち、まず、バイアル瓶内に注射用蒸留水等を１ml加え、次いで、内容物を均一に溶解して使用する。安定性に優れ、有効成分として本発明による２４種類のポリペプチドを含んでなる本品は、スギ花粉症を治療・予防するための乾燥製剤として有用である。

【０１２６】製剤例３.

錠剤

平均分子量約20,000ダルトンの精製プルラン２g を蒸留水１００mlに均一に溶解し、溶液に塩化シアヌルの1.7％(w/v) アセトン溶液を２ml加え、５％(w/v)炭酸ナトリウム水溶液でpHを７付近に保ちつつ、撹拌下、５℃で

２時間反応させた。その後、同様にして反応物のpHを７付近に保ちながら、４℃の冷水に対して一晩透析し、活性化プルランを含む水溶液２０mlを得た。

【０１２７】実施例１乃至２４記載の方法により得たペプチドをそれぞれ0.2mg加え、溶液のpHを７付近に保ちつつ、穏やかに撹拌しながら、３７℃で１２時間反応させた。反応後、反応物にグリシンを４ｇを加え、穏やかに撹拌しながら、３７℃で５時間インキュベートし、未反応の活性基をブロックした。反応物を濃縮し、あらかじめ0.1Ｍ リン酸緩衝液（pH 7.0）で平衡化させておいたセファデックス G-50 カラムに供与し、カラムに新鮮な同一緩衝液を通液して、この発明のペプチドとプルランの複合体を含む画分を採取した。収量は、原料ペプチド固形分当たり、約３０％であった。

【０１２８】常法に従って、この画分を滅菌濾過し、濃縮し、凍結乾燥し、粉砕後、マンニトールを均一に混合し、混合物を打錠して製品１錠（200mg）当たり複合体を２、１０又は５０mg含む錠剤を得た。摂取性、安定性に優れた本品は、スギ花粉症を治療・予防するための舌下剤として有用である。

【０１２９】製剤例４.

シロップ剤

大腸菌由来の精製リボ多糖１ｇを１０mMリン酸カルシウム溶液１００mlに溶解し、溶液に１００mM過ヨウ素酸ナトリウムを６ml加え、室温下で２０分間反応させてリボ多糖を活性化した。反応物を４℃の１Ｍ グリシン－塩酸緩衝液（pH 4.4）に対して一晩透析して未反応の過ヨウ素酸を除去した後、0.1Ｍ 炭酸水素ナトリウム緩衝液によりpH 9.5付近に調整する一方、別途、実施例１乃至２４記載の方法により得た２４種類のペプチドを0.1Ｍ リン酸緩衝液（pH 7.0）１００mlにそれぞれ１０mgずつ溶解し、活性化リボ多糖を含む上記反応物に加え、室温下で１２時間静置して反応させた。

【０１３０】その後、新たに得られた反応物を製剤例３の方法により精製し、得られた本発明のペプチドとリボ多糖の複合体を含む画分を濃縮し、凍結乾燥し、粉砕して固状物とした。収量は、原料ペプチド固形分当たり、約３０％であった。この固形物を蔗糖をそれぞれ最終濃度が0.1若しくは1 mg/ml 又は５０％(w/w) になるように安定剤として精製ゼラチンを１％(w/w) 含む蒸留水に溶解し、溶液を常法により滅菌濾過してシロップ状物を得た。このシロップ状物を２mlずつ滅菌バイアル瓶に分注し、密栓して製品とした。安定性に優れ、有効成分としてこの発明のペプチドとリボ多糖の複合体を含む本品は、スギ花粉症を治療・予防するためのシロップ剤として有用である。

【０１３１】

【発明の効果】本発明により、スギ花粉アレルゲンのＴ細胞エピトープのみからなるペプチド及びそれらを有効成分として含んでなる抗スギ花粉症剤を提供することができた。そして、本発明のペプチドは、スギ花粉アレルゲンに特異的なイムノグロブリンＥ抗体に実質的に反応しないので、ヒトを含む哺乳類一般に投与すると、実質的にアナフィラキシーを引起こすことなく、スギ花粉アレルゲンに特異的なＴ細胞を活性化することができる。

【０１３２】

【配列表】

配列番号：１
配列の長さ：１４
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser
1 5 10

【０１３３】配列番号：２
配列の長さ：１３
配列の型：アミノ酸
配列
Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser
1 5 10

【０１３４】配列番号：３
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser
1 5 10

【０１３５】配列番号：４
配列の長さ：１４
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド

35 36

Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly
1 5 10

【０１３６】配列番号：５
配列の長さ：１３
配列の型：アミノ酸
配列
Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met
1 5 10

【０１３７】配列番号：６
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu
1 5 10

【０１３８】配列番号：７
配列
His Phe Thr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln
1 5 10

【０１３９】配列番号：８
配列の長さ：１４
配列の型：アミノ酸
配列
Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe
1 5 10

【０１４０】配列番号：９
配列の長さ：１１
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser
1 5 10

【０１４１】配列番号：１０
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列
Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu
1 5 10

【０１４３】配列番号：１２
配列の長さ：１１
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu
1 5 10

【０１４４】配列番号：１３
配列の長さ：１４
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met
1 5 10

【０１４５】配列番号：１４
配列の長さ：１０

配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu
1 5 10

【０１４６】配列番号：１５
配列の長さ：１１
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met
1 5 10

【０１４７】配列番号：１６
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly
1 5 10

【０１４８】配列番号：１７
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn
1 5 10

【０１４９】配列番号：１８
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr
1 5 10

【０１５０】配列番号：１９
配列の長さ：１１
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn
1 5 10

【０１５１】配列番号：２０
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu
1 5 10

【０１５２】配列番号：２１
配列の長さ：１１
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu
1 5 10

【０１５３】配列番号：２２
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn
1 5 10

【０１５４】配列番号：２３
配列の長さ：１１
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn
1 5 10

【０１５５】配列番号：２４
配列の長さ：１２
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：ペプチド
配列
Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp
1 5 10

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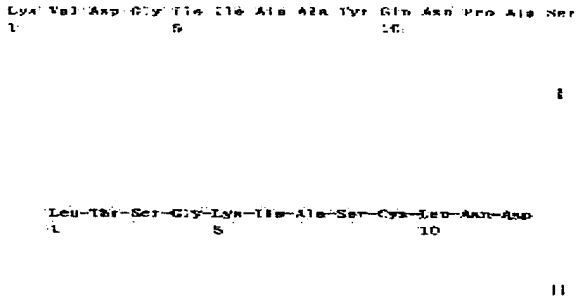
(54) PEPTIDE AND ITS USE

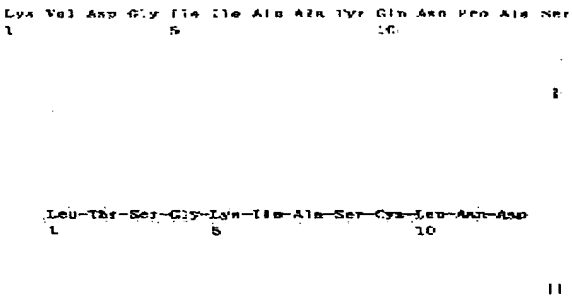
(57)Abstract:

PURPOSE: To obtain a new peptide not reacting with immunoglobulin E antibody specific to cedar pollen antigen, not causing anaphylaxis, capable of activating T-cells specific to the cedar pollen antigen, and useful for cedar pollinosis medicines.

CONSTITUTION: A peptide comprising



amino acid sequences of formula I and II, etc. The peptide is obtained e.g. by setting Fmoc-L-amino acid Wang resin, into which an amino acid corresponding to the C-terminal of the peptide to be synthesized is introduced, to the reactor of a peptide-synthesizing device, removing the Fmoc with a deprotection solution, further reacting an activator solution with an amino acid solution corresponding to the second amino acid from the C terminal, again performing the deprotection of the Fmoc group, and similarly repeating the same operations.



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* NOTICES *

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1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The peptide which consists of the amino acid sequence of the array number 1.
[Claim 2] The peptide which consists of the amino acid sequence of the array number 2.
[Claim 3] The peptide which consists of the amino acid sequence of the array number 3.
[Claim 4] The peptide which consists of including the amino acid sequence of the array number 3.
[Claim 5] The peptide which consists of the amino acid sequence of the array number 4.
[Claim 6] The peptide which consists of the amino acid sequence of the array number 5.
[Claim 7] The peptide which consists of the amino acid sequence of the array number 6.
[Claim 8] The peptide which consists of including the amino acid sequence of the array number 6.
[Claim 9] The peptide which consists of the amino acid sequence of the array number 7.
[Claim 10] The peptide which consists of including the amino acid sequence of the array number 7.
[Claim 11] The peptide which consists of the amino acid sequence of the array number 8.
[Claim 12] The peptide which consists of including the amino acid sequence of the array number 8.
[Claim 13] The peptide which consists of the amino acid sequence of the array number 9.
[Claim 14] The peptide which consists of including the amino acid sequence of the array number 9.

[Claim 15] The peptide which consists of the amino acid sequence of the array number 10.
[Claim 16] The peptide which consists of the amino acid sequence of the array number 11.
[Claim 17] The peptide which consists of the amino acid sequence of the array number 12.
[Claim 18] The peptide which consists of including the amino acid sequence of the array number 12.
[Claim 19] The peptide which consists of the amino acid sequence of the array number 13.
[Claim 20] The peptide which consists of the amino acid sequence of the array number 14.
[Claim 21] The peptide which consists of including the amino acid sequence of the array number 14.
[Claim 22] The peptide which consists of the amino acid sequence of the array number 15.
[Claim 23] The peptide which consists of the amino acid sequence of the array number 16.
[Claim 24] The peptide which consists of the amino acid sequence of the array number 17.
[Claim 25] The peptide which consists of including the amino acid sequence of the array number 17.
[Claim 26] The peptide which consists of the amino acid sequence of the array number 18.
[Claim 27] The peptide which consists of the amino acid sequence of the array number 19.
[Claim 28] The peptide which consists of including the amino acid sequence of the array number 19.
[Claim 29] The peptide which consists of the amino acid sequence of the array number 20.
[Claim 30] The peptide which consists of including the amino acid sequence of the array number 20.
[Claim 31] The peptide which consists of the amino acid sequence of the array number 21.
[Claim 32] The peptide which consists of including the amino acid sequence of the array number 21.
[Claim 33] The peptide which consists of the amino acid sequence of the array number 22.
[Claim 34] The peptide which consists of the amino acid sequence of the array number 23.
[Claim 35] The peptide which consists of including the amino acid sequence of the array number 23.

[Claim 36] The peptide which consists of the amino acid sequence of the array number 24.
[Claim 37] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 1.
[Claim 38] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 2.
[Claim 39] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 3.
[Claim 40] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 3.
[Claim 41] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 4.
[Claim 42] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 5.
[Claim 43] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 6.
[Claim 44] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 6.
[Claim 45] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 7.
[Claim 46] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 7.
[Claim 47] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 8.
[Claim 48] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 8.
[Claim 49] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 9.
[Claim 50] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 9.
[Claim 51] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 10.
[Claim 52] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 11.
[Claim 53] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 12.
[Claim 54] The anti-hay fever agent which makes an active principle the

peptide which consists of including the amino acid sequence of the array number 12.
[Claim 55] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 13.
[Claim 56] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 14.
[Claim 57] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 14.
[Claim 58] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 15.
[Claim 59] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 16.
[Claim 60] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 17.
[Claim 61] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 17.
[Claim 62] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 18.
[Claim 63] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 19.
[Claim 64] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 19.
[Claim 65] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 20.
[Claim 66] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 20.
[Claim 67] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 21.
[Claim 68] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 21.
[Claim 69] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 22.
[Claim 70] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 23.
[Claim 71] The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 23.

[Claim 72] The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 24.

[Translation done.]

JP08-3333-91

* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the peptide which activates the T cell which reacts to cedar pollen allergen specifically, and the immunotherapy agent which comes to contain that peptide as an active principle.
[0002]
[Description of the Prior Art] If it has become at the beginning of spring in our country since here dozens years, the number of those who appeal against the rhinitis and the conjunctivitis by hay fever will continue increasing. Hay fever is a kind of ARUREGI ** and it is said that the main factor is, the antigenic matter, i.e., the hay fever allergen, in cedar pollen. If the cedar pollen which dispersed in atmospheric air trespasses upon the human inside of the body, the immunoglobulin E antibody to cedar pollen allergen will produce. When cedar pollen invades next in this condition, the allergen and this immunoglobulin E antibody in that pollen will cause an immunoreaction, and will present an allergy symptom.
[0003] It is known by current that at least two kinds of allergen to which antigenic is different in cedar pollen exists. One of them is allergen which YASUEDA etc. has reported to "journal OBU allergy - and - clinical immunology", the 71st volume, No. 1, and the 77-86th page (1983), and this is called "Cryj1" today. In addition, Cryj1 International application of the overall-length amino acid sequence is determined and carried out (WO 93/01213).
[0004] Another is TANIAI, etc. the 239th volume "EFU I BI S Letters", No. 2, the 329-332nd page (1988), Sakaguchi, etc. No. "allergy" 45, and allergen reported to the 309-312nd page (1990), and this is called "Cry j 2" today. In addition, Cry j The overall-length amino acid sequence is determined, and international application of 2 is carried out (WO 94/11512). moreover, Komiyama ** — although the overall-length amino acid sequence of Cryj2 is determined separately (Biochem.Biophys.Res, Comm., vol.201, No.2, and 1021-1028 (1994)) — WO Four amino acid residue differs from the amino acid sequence of 94/11512 publication.
[0005] Into cedar pollen, Cryj1 and Cryj2 exist at a rate of about 50:1 thru/or 5:1, and they are usually said for most blood serums extracted from the hay fever sufferer to react to Cryj1 and Cryj2. Sawatani and others has reported that Cryj2 demonstrates antigenic [comparable as Cryj1] in an intracutaneous-reaction trial or a RAST trial in "allergy", the 42nd volume, No. 6, and the 738-747th page (1993).
[0006] Thus, since cedar pollen allergen was already isolated partly and the property and description were also solved to some extent, the prospect which can treat and prevent hay fever followed by prescribing for the patient and carrying out hyposensitization of the purification cedar pollen allergen to Homo sapiens. Recently, it succeeds in the proposal with which carries out covalent bond of the sugar to the cedar pollen allergen as which some hyposensitization agents for it are also devised, for example, the amino acid sequence from an amino terminal is expressed in Asp-Asn-Pro-Ile-Asp-Ser or Ala-Ile-Asn-Ile-Phe-Asn to JP,1-156926,A or JP,3-93730,A, and Homo sapiens is medicated by making the generated complex into a hyposensitization agent.
[0007] However, the allergen of a high grade is needed in large quantities, and if the allergen in cedar pollen has low stability to a small top and it is going to provide the diagnostic agent and hyposensitization agent of hay fever only with cedar pollen to it, great difficulty will usually follow it on a diagnosis and desensitization therapy of an allergy. Since it is such, in the therapy and prevention of the latest allergosis, like the former, a patient is not medicated with the whole allergen but the minimum area which the T cell in allergen recognizes specifically, i.e., the immunotherapy which prescribes for the patient the low-molecular peptide which essentially consists only of a T cell epitope, attracts attention.
[0008] Generally, when allergen is incorporated by antigen presenting cells, such as a macrophage, it is digested there, and a digestive fragment will join together and antigen presentation will be carried out to the HLA (Human Leucocyte Antigen) protein of an immunity presentation cell cortex. The field which the fragment by which antigen presentation is carried out is restricted to some [in allergen] specific regions with the

compatibility over HLA protein etc., and a T cell recognizes specifically among these fields is usually called a "T cell epitope." In the immunotherapy which prescribes for the patient the peptide which consists only of T cell EPUTOPU substantially [0009] (i) The peptide lacks the B cell epitope, namely, since a specific immunoglobulin E antibody does not react to allergen, side effects, such as anaphylaxis which had occurred frequently with the conventional poor quality or purification allergen, cannot happen.
(ii) It starts from small quantity and a period until it reaches an effective dose can be sharply shortened as compared with the conventional hyposensitization agent.
(iii) Peroral immunity tolerance can be guided and the allergic response to allergen can be decreased. There is which advantage.
[0010]
[Problem(s) to be Solved by the Invention] this invention persons completed a header and this invention for the amino acid sequence of the smallest unit which constitutes the above-mentioned T cell epitope. The first technical problem of this invention is to offer the peptide which consists only of a T cell epitope of an essential target's cedar pollen allergen. The second technical problem of this invention is to offer the anti-hay fever agent which comes to contain the above-mentioned peptide as an active principle.
[0011]
[Means for Solving the Problem] This invention (1) The peptide which consists of the amino acid sequence of the array number 1, (2) Peptide which consists of the amino acid sequence of the array number 2 (3) The peptide which consists of the amino acid sequence of the array number 3, (4) The peptide which consists of including the amino acid sequence of the array number 3, (5) Peptide which consists of the amino acid sequence of the array number 4 (6) The peptide which consists of the amino acid sequence of the array number 5, (7) Peptide which consists of the amino acid sequence of the array number 6 (8) Peptide which consists of including the amino acid sequence of the array number 6 (9) The peptide which consists of the amino acid sequence of the array number 7, peptide which consists of including the amino acid sequence of (10) array number 7, [0012] (11) The peptide which consists of the amino acid sequence of the array number 8, the peptide which consists of including the amino acid sequence of (12) array number 8, (13) The peptide which consists of the amino acid sequence of the array number 9, the peptide which consists of including the amino acid sequence of (14) array number 9, (15) The peptide which consists of the amino acid sequence of the array number 10, the peptide which consists of the amino acid sequence of (16) array number 11, (17) The peptide which consists of the amino acid sequence of the array number 12, the peptide which consists of including the amino acid sequence of (18) array number 12, the peptide which consists of the amino acid sequence of (19) array number 13, peptide which consists of the amino acid sequence of (20) array number 14, [0013] (21) The peptide which consists of including the amino acid sequence of the array number 14, (22) The peptide which consists of the amino acid sequence of the array number 15, the peptide which consists of the amino acid sequence of (23) array number 16, (24) The peptide which consists of the amino acid sequence of the array number 17, the peptide which consists of including the amino acid sequence of (25) array number 17, (26) The peptide which consists of the amino acid sequence of the array number 18, the peptide which consists of the amino acid sequence of (27) array number 19, (28) The peptide which consists of including the amino acid sequence of the array number 19, the peptide which consists of the amino acid sequence of (29) array number 20, the peptide which consists of including the amino acid sequence of (30) array number 20, [0014] (31) The peptide which consists of the amino acid sequence of the array number 21, the peptide which consists of including the amino acid sequence of (32) array number 21, (33) The peptide which consists of the amino acid sequence of the array number 22, the peptide which consists of the amino acid sequence of (34) array number 23, (35) The peptide which consists of including the amino acid sequence of the array number 23, (36) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 24, and the peptide which consists of the amino acid sequence of (37) array number 1, (38) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 2, (39) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 3, anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of (40) array number 3, [0015] (41) an array — a number — four — an amino acid sequence — from — change — a peptide — an active principle — ** — carry out — anti- — hay fever — an agent — (— 42 —) — an array — a number — five — an amino acid sequence — from — change — a peptide — an active principle — ** — carry out — anti- — hay fever — an agent — (— 43 —) — an array — a number — six — an amino acid sequence — from — change — a peptide — an active principle — ** — carry out — anti- — hay fever — an agent — (— 44 —) — an array — a number — six — an amino acid sequence — contain — things — from — change — a peptide — an active principle — ** — carry out — anti- — hay fever — an agent — (— 45 —) — an array — number — seven — an amino acid sequence — from — change — a peptide — an active principle — ** — carry out — anti- — hay fever — an agent [0016] (46) The anti-hay fever agent which

makes an active principle the peptide which consists of including the amino acid sequence of the array number 7, (47) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 8, (48) The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 8, (49) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 9, anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of (50) array number 9, [0017] (51) an array -- a number -- ten -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 52 --) -- an array -- a number -- 11 -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 53 --) -- an array -- a number -- 12 -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 54 --) -- an array -- a number -- 12 -- an amino acid sequence -- contain -- things -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 55 --) -- an array -- number -- 13 -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent [0018] (56) an array -- a number -- 14 -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 57 --) -- an array -- a number -- 14 -- an amino acid sequence -- contain -- things -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 58 --) -- an array -- a number -- 15 -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 59 --) -- an array -- a number -- 16 -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent -- (-- 60 --) -- an array -- number -- 17 -- an amino acid sequence -- from -- change -- a peptide -- an active principle -- ** -- carry out -- anti- -- hay fever -- an agent [0019] (61) The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 17, (62) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 18, (63) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 19, (64) The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 19, anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of (65) array number 20, [0020] (66) The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 20, (67) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 21, (68) The anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 21, (69) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 22, (70) The anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of the array number 23, (71) It is related with the anti-hay fever agent which makes an active principle the peptide which consists of including the amino acid sequence of the array number 23, and the anti-hay fever agent which makes an active principle the peptide which consists of the amino acid sequence of (72) array number 24.

[0021] Hereafter, this invention is explained in detail. The example of the desirable peptide in this invention is as in Table 1.

[0022]

[Table 1]

---------- (1) Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser (peptide 1)

(2) Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser (peptide 2)

(3) Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser (peptide 3)

(4) Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly (peptide 4)

(5) Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met (peptide 5)

(6) Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu (peptide 6)

(7) His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln (peptide 7)

(8) Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe (peptide 8)

(9) Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser (peptide 9)

(10) Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp (peptide 10)

(11) Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu (peptide 11)

(12) Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu (peptide 12)

(13) Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met (peptide 13)

(14) Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu (peptide 14)

(15) Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met (peptide 15)
(16) Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly (peptide 16)
(17) Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn (peptide 17)
(18) Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr (peptide 18)
(19) Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn (peptide 19)
(20) Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu (peptide 20)
(21) Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu (peptide 21)
(22) Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn (peptide 22)
(23) Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn (peptide 23)
(24) Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp (peptide 24)

[0023] The above-mentioned peptide 1 in addition, the peptide shown according to the amino acid sequence of the array number 1 of an array table and the above-mentioned peptide 2 The peptide shown according to the amino acid sequence of the array number 2 of an array table, and the above-mentioned peptide 3 The peptide shown according to the amino acid sequence of the array number 3 of an array table, and the above-mentioned peptide 4 The peptide shown according to the amino acid sequence of the array number 4 of an array table, and the above-mentioned peptide 5 The peptide shown according to the amino acid sequence of the array number 5 of an array table, and the above-mentioned peptide 6 For the peptide shown according to the amino acid sequence of the array number 6 of an array table, and the above-mentioned peptide 7, the peptide shown according to the amino acid sequence of the array number 7 of an array table and the above-mentioned peptide 8 are a peptide shown according to the amino acid sequence of the array number 8 of an array table, [0024] The above-mentioned peptide 9 the peptide shown according to the amino acid sequence of the array number 9 of an array table, and the above-mentioned peptide 10 The peptide shown according to the amino acid sequence of the array number 10 of an array table, and the above-mentioned peptide 11 The peptide shown according to the amino acid sequence of the array number 11 of an array table, and the above-mentioned peptide 12 The peptide shown according to the amino acid sequence of the array number 12 of an array table, and the above-mentioned peptide 13 For the peptide shown according to the amino acid sequence of the array number 13 of an array table, and the above-mentioned peptide 14, the peptide shown according to the amino acid sequence of the array number 14 of an array table and the above-mentioned peptide 15 are a peptide shown according to the amino acid sequence of the array number 15 of an array table, [0025] The above-mentioned peptide 16 the peptide shown according to the amino acid sequence of the array number 16 of an array table, and the above-mentioned peptide 17 The peptide shown according to the amino acid sequence of the array number 17 of an array table, and the above-mentioned peptide 18 The peptide shown according to the amino acid sequence of the array number 18 of an array table, and the above-mentioned peptide 19 The peptide shown according to the amino acid sequence of the array number 19 of an array table, and the above-mentioned peptide 20 The peptide shown according to the amino acid sequence of the array number 20 of an array table, and the above-mentioned peptide 21 The peptide shown according to the amino acid sequence of the array number 21 of an array table, and the above-mentioned peptide 22 The peptide in which the peptide shown according to the amino acid sequence of the array number 22 of an array table and the above-mentioned peptide 23 are shown according to the amino acid sequence of the array number 23 of an array table, and the above-mentioned peptide 24 express the peptide shown according to the amino acid sequence of the array number 24 of an array table, respectively.

[0026] A peptide the above (1) thru/or given in (36) can be easily prepared with the peptide synthesis method of common use in the field known as a "solid phase technique" or a "liquid phase process." For example, the detail of peptide synthesis is indicated by the Tokyo Kagaku Dojin issue in the edited by Japanese Biochemical Society "a new chemistry experiment lecture", the 1st volume, "protein VI", the 3-44th page, and 1992. Moreover, this peptide is a multi-peptide synthesizer. SYMPHONY (pro TIN technology company make) is used, and it is Fmoc. (9-fluorenyl methyloxycarbonyl) According to the protocol of this equipment, it is compoundable with a solid phase synthesis method. That is, the amino acid equivalent to the C terminal of each peptide to compound is introduced. Fmoc-L-amino acid Wang Resin is set to the reaction container of the above-mentioned peptide synthesizer unit, and a deprotection solution is used. Fmoc It removes. the amino acid solution and activator solution which are furthermore equivalent to the 2nd amino acid from a C terminal are reacted — making — after a reaction — again — Fmoc The target peptide is compoundable by performing deprotection of a radical and repeating the same actuation.

[0027] The peptide of this invention is not limited to what was prepared by chemosynthesis. For example, the cedar pollen allergen which extracted from the pollen or the male of a Japan cedar, or was prepared by recombinant DNA technology is decomposed suitably. DNA which carries out the code of the peptide which could extract from the decomposition product, for example, was indicated by the above (1) thru/or (36) is prepared. It inserts in the vector which can replicate this autonomously and considers as a recombinant DNA,

and Escherichia coli, a Bacillus subtilis, an Actinomyces, yeast, etc. may introduce this into a host suitably, it may consider as a transformant, and the peptide of this invention may be extracted from that culture.
[0028] Furthermore, the peptide of this invention may be a gestalt as the gestalt, the derivative which is made to carry out the bridge formation polymerization of the peptide by acetylation, amidation, and/or polyfunctional trial, and is obtained further, or polymer as complex which adds sugar and a polyethylene glycol to the peptide obtained thus, and is obtained.
[0029] The peptide of this invention is usually refined in advance of use, although expected therapy and preventive effect are demonstrated even if it prescribes a medicine for the patient with a comparatively **** gestalt. What is necessary is to use the approach of the common use in the field for refining a peptide thru/or protein, such as filtration, concentration, centrifugal separation, gel filtration chromatography, an ion exchange chromatography, a high speed liquid chromatography, affinity chromatography, gel electrophoresis, and isoelectric focusing, for purification, and just to combine these approaches with it suitably if needed. And what is necessary is to condense the refined peptide, to freeze-dry according to an end-use gestalt, and just to make it liquefied or a solid state.
[0030] It is a T cell specific to cedar pollen allergen that the peptide of this invention has the activity as a T cell epitope. It can check by measuring the incorporation of 3H-thymidine. The following approaches can be used for this measurement. namely, the mononuclear cell group which contains a specific T cell in Cryj2 from laboratory animals, such as a mouse which carried out immunity by a hay fever sufferer's peripheral blood or Cryj2 by the Ficoll-Hypaque-gradient-centrifugation method etc., — dissociating — this cell population — RPMI 1640 etc. — a culture medium is made to float and it pours distributively on 96 well microplate. Next, the peptide which is a specimen material is added and it incubates. Although the temperature and time amount of this incubation can be suitably adjusted for every experiment, 37 degrees C and two days are suitable. After that 3H-thymidine is added to a culture medium, a fixed time amount incubation is continued further, and it can set in a mononuclear cell group. By measuring the amount of incorporation of 3H-thymidine, the activity as a T cell epitope of the peptide of this invention is reckonable. in addition, in this invention, the system which does not contain a peptide in coincidence is prepared and let this be a negative control — the system to which the amount of incorporation of 3H-thymidine reached the more than twice of a negative control was made into the "positivity", and the system which was not attained was made "negative."
[0031] T cell specific to cedar pollen allergen Measurement of the incorporation of 3H-thymidine can be performed also by the following approaches. Immunity of the laboratory animals, such as a mouse, is beforehand carried out by Cryj2, and a lymphocyte is extracted from submandibular lymph nodes etc. after that. then, it stimulates by the same approach as the above with the peptide which is analyte -- the activity as a T cell epitope of the peptide of this invention is reckonable by measuring the amount of incorporation of 3H-thymidine. The judgment electropositive [of a peptide / "electropositive"] and "negative" was performed on the same criteria as the above.
[0032] It can check by the following experiments that the peptide of this invention has a preventive effect in a hay fever sufferer. The peptide of this invention is beforehand prescribed for the patient to laboratory animals, such as a mouse, and the immunological tolerance to this peptide is guided. Immunity of Cryj2 is prescribed for the patient and carried out to the laboratory animal concerned with adjuvants, such as a cholera toxin, after fixed period progress. Furthermore, after fixed period progress, from the laboratory animal concerned, a submandibular-lymph-nodes cell is extracted and cell suspension is prepared.
[0033] Moreover, from the laboratory animal which is not processed [different from this], a spleen is extracted, spleen cell suspension is prepared, an X-ray is irradiated at this, and cell proliferation activity is vanished, and let this be antigen presenting cell content suspension. This thing is mixed with previous submandibular-lymph-nodes cell suspension, Cryj2 is added to this, culture is continued, and it is a pan. 3H-thymidine can be added, incorporation of this thing can be measured, and growth of a T cell can be measured.
[0034] Beforehand, with the peptide of this invention, for the animal which is not guiding immunological tolerance, it reacts to Cryj2 which the T cell combined with the antigen presenting cell by immunization by Cryj2, and increases. On the other hand, for the animal which guided immunological tolerance with the peptide of this invention beforehand, even if it performs immunity by Cryj2 after that, a T cell does not react to Cryj2 combined with the antigen presenting cell, and it does not increase. By measuring the difference, the preventive effect over the pollinosis of the peptide of this invention can be checked.
[0035] Furthermore, although the cytokine of interleukin 4 grade is secreted in culture medium when Cryj2 is added into the submandibular-lymph-nodes cell suspension of an above-mentioned immune animal, and the mixed liquor of antigen presenting cell content suspension and culture is continued, the preventive effect over the pollinosis of the peptide of this invention can be checked also by measuring the amount of secretion of this cytokine by the laboratory animal which front-prescribed the peptide of this invention for the patient, and performed tolerance induction, and the laboratory animal which was not front-prescribed for the patient.

[0036] It can check by the following experiments that the peptide of this invention has a curative effect in a hay fever sufferer. Immunity of Cryj2 is beforehand prescribed for the patient and carried out with the AJU band of a cholera toxin to laboratory animals, such as a mouse. The booster of Cryj2 is prescribed for the patient and carried out to the laboratory animal concerned with the AJU band of a cholera toxin after fixed period progress. Furthermore, after extracting a submandibular-lymph-nodes cell and preparing cell suspension from the laboratory animal concerned after fixed period progress, growth of a T cell is measured by the same approach as the above.
[0037] For the animal which has not treated with the peptide of this invention, it reacts to Cryj2 which the T cell combined with the antigen presenting cell according to the immunity by Cryj2, and increases. On the other hand, for the animal treated with the peptide of this invention, even if it performs immunity by Cryj2 after that, a T cell does not react to Cryj2 combined with the antigen presenting cell, and it does not increase. By measuring the difference, the curative effect over the pollinosis of the peptide of this invention can be checked.
[0038]
[work —] for The peptide of this invention can activate a specific T cell to cedar pollen allergen, without causing anaphylaxis substantially, if the general mammals including Homo sapiens are medicated since it does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially. If the general mammals including Homo sapiens are medicated with the anti-hay fever agent of this invention which comes to contain this peptide as an active principle, it will demonstrate remarkable therapy and preventive effect to hay fever, without causing anaphylaxis substantially.
[0039] The anti-hay fever agent which comes to contain the peptide of this invention as an active principle can treat hay fever, without causing side effects, such as anaphylaxis, substantially, if the general mammals which is suffered from hay fever and contains Homo sapiens are medicated. When medicating a healthy individual and the individual of potential hay fever with the example of anti-hay fever of this invention before cedar pollen begins to disperse, while demonstrating a remarkable preventive effect to hay fever on the other hand, higher efficacy is demonstrated to the remission of the allergy symptom at the time of the onset.
[0040] one sort of the peptide usually according [the anti-hay fever agent of this invention] to this invention if it explains to the anti-hay fever agent per pan of this invention in detail, or two sorts or more — 0.01 thru/or 100% (w/w) — desirable — 0.05 thru/or 50% (w/v) — further — desirable — 0.5 thru/or 5.0% (w/w) It comes to contain. A gestalt peptide independent [concerned] is permitted physiologically except that from the first, for example, the anti-hay fever agent of this invention includes the gestalt as a constituent with support, such as serum albumin, gelatin, and a mannitol, an excipient, an immunoadjuvant, a stabilizer, one sort containing anti-inflammatory agents and antihistamines, such as steroid hormone and chestnut MOGURIKU acid sodium, or two sorts or more of other drugs further if needed. Furthermore, the anti-hay fever agent of this invention also includes the drugs of medication unit form voice, and the drugs of that medication unit form voice contain the amount which is equivalent to the dosage per day, its integral multiple (up to 4 times), or its divisor in the polypeptide of this invention (to 1/40), and mean the drugs in the dosage forms of one suitable for administration separated physically. As drugs of such medication unit form voice, powder, a fine grain agent, a granule, a pill, a tablet, a capsule, the trochiscus, syrups, an emulsion, a mild steel agent, plaster, cataplasms, suppositories, ophthalmic solutions, a nasal drop, a spray, injections, etc. are mentioned.
[0041] the general mammals in which the anti-hay fever agent of this invention contains Homo sapiens for the purpose of the therapy and prevention of hay fever when the operation of the anti-hay fever agent of this invention is explained — transderma, taking orally, and the rhinenchysis — a medicine is applied eyewash or injection prescribed for the patient although the dose in Homo sapiens changes even if it depends on the purpose and symptom of administration, while usually observing a candidate's symptom and the progress after administration — an adult — per [0.01] day thru/or 1.0g — desirable — 0.01 thru/or 0.1g a standard — 1 time of 1 time of every week thru/or every month of frequency — it is — about 1 — or repeated-dose administration is usually carried out for six months, increasing a dosage.
[0042] the immunity therapy agent obtained to the mouse on after-the-birth the 20th the acute toxicity conventional method of the polypeptide of this invention by the below-mentioned example 1 of pharmaceutical preparation thru/or the approach of 4 — taking orally — or it injected intraperitoneally. Consequently, these immunotherapy agents are 200 mg/kg by which route of administration. It became clear that it was the above fifty percent lethal dose. This shows that combination use can be carried out to insurance at the immunotherapy agent to the mammals in which the peptide of this invention contains Homo sapiens.
[0043] It checked that the peptide 1 thru/or the peptide 6 and the peptide 9 thru/or peptide 24 of this invention had cedar pollen antigen T cell epitope activity using the T cell isolated from the example of trial 1. hay fever patient. In a skin test, a positivity is shown to cedar pollen allergen, and it is anti-cedar pollen allergen. IgE 20ml peripheral blood was extracted from the patient who shows a positivity to a reaction. The buffy coat was obtained after centrifugal separation and the peripheral blood monocyte (Peripheral Blood

Mononuelear Cells:PBMC) was further extracted by the ficoll pack specific gravity centrifuge method. To a culture medium (RPMI-1640 and 5% of heat inactivation Homo sapiens AB mold blood serum are included.), it is this PBMC 7.5x105 It suspended so that it might be set to a cell/ml.

[0044] It sets on the circular plate of 96 wells, and is 1.5x105. About a cell, it is each well 200microl. They are the peptide of 20ng(s), and 37-degree-C5%CO2 in a culture medium. It cultivated under existence for 48 hours. Then, 1microcurie tritition thymidine was added and it cultivated for further 16 hours. In order to measure the count incorporated by the cell, cells were collected on the glass fiber filter using the cell harvester, and it measured with the liquid scintillation counter. This result is shown in the following table 2.

[0045]

[Table 2]

---------- A peptide T cell epitope activity ----------

The peptide 1 Positive Sex Peptide 2 Positive Sex Peptide 3 Positive Sex Peptide 4 positive Sex Peptide 5 Positive Sex Peptide 6 Positive Sex Peptide 9 Positive Sex Peptide 10 Positive Sex Peptide 11 Positivity Peptide 12 Positive Sex Peptide 13 Positive Sex Peptide 14 Positive Sex Peptide 15 Positive Sex Peptide 16 Positive Sex Peptide 17 Positive Sex Peptide 18 positive Sex Peptide 19 Positive Sex Peptide 20 positive Sex Peptide 21 Positive Sex Peptide 22 Positive Sex Peptide 23 Positive Sex Peptide 24 positive From the result more than sex ---------- ----------, it was shown that these peptides contain the T cell epitope of Cryj2 allergen.

[0046] Approach given [example of trial 2.Cryj2] in reference (Allergy, 1990, 45, 309-312) It refined. Refined Cryj2 1microg It is 0.01M about cholera toxin B subunit 1microg (0.5% content of cholera toxins). Phosphate buffer solution (pH 7.4) About the antigen solution in which it was made to dissolve, it is under the Ava Ching anesthesia. Balb/c Rhinenchysis administration was carried out and immunity was carried out to the mouse (5-6 weeks old: Charles RIBAJAPAN). The booster of this mouse was again carried out by the same approach after the two weeks. The submandibular-lymph-nodes cell of a mouse was extracted after the one week. It let this pass to the nylon mesh, it suspended further in the culture medium (RPMI 1640 10% calf embryo blood serum content), and suspension was prepared.

[0047] Moreover, from the mouse which has not been immunized by Cryj2, the spleen cell was extracted and lymph gland cell suspension was prepared by the same approach as the above. The X-ray of 3000 Rad was irradiated at this suspension, the growth activity of a cell was vanished, and it used as antigen presenting cell suspension. To a flat bottom 96 well plate (Corning, Inc.), they are the lymph gland cell 3x106 and an antigen presenting cell 6x105 per one well. It pours distributively so that it may become, and they are 37 degrees C and 5%CO2 under existence of a peptide 7 or a peptide 8 or the nonexistence (0.5microg/(ml)) of these peptides. It cultivated for bottom three days of a condition.

[0048] The last 16 hours and 3 H-Thymidine It cultivated under existence and was incorporated in [DNA] the nucleus in the meantime. 3 H-Thymidine It calculated by measuring the dosage of DNA which adsorbed the amount at the glass filter by the liquid scintillation method. Under peptide existence 3 H-Thymidine This was made into the index of cell proliferation activity by making into a reaction scale factor the value which broke the amount of incorporation by the amount of incorporation under peptide nonexistence.

[0049] To the peptide 7, as for the lymph gland cell, the reproductive rate increased by about 5 times to about 3 times and a peptide 8. Therefore, it was shown that these peptides contain the T cell epitope of Cryj2 allergen.

[0050] About the example of trial 3. peptide 7, or 8, it is Balb/c. Immunological tolerance was guided to the mouse. Namely, phosphate buffer solution (0.01M (pH 7.4)) About each peptide solution in which it was made to dissolve, it is 20microg per animal to a mouse caudal vein. Vein administration was performed so that it might become the amount of peptides. Or this peptide solution was administered orally so that it might become the one-animal amount of peptides of 1mg per time, and this internal use was repeated 4 times at two weeks. Then, immunity by Cryj2 was performed by the same approach as the example 2 of a trial about the mouse concerned.

[0051] Extract a submandibular-lymph-nodes cell from this mouse by the same approach as the example 2 of a trial, and it considers as submandibular-lymph-nodes cell suspension. A spleen is extracted from the separate mouse which omits tolerant-izing by the peptide, and immunity induction by Cryj2, and growth activity is vanished with an X-ray. Moreover, as antigen presenting cell suspension These are cocultivated under existence (1microg/(ml)) of Cryj2, and it is by the same approach as the example 2 of a trial. 3 H-Thymidine The amount of incorporation was measured and cell proliferation activity was calculated.

[0052] Moreover, the suspension of a lymph gland cell and an antigen presenting cell was prepared by the preparation culture medium. They are the lymph gland cell 1.5x106 and an antigen presenting cell 3x106 per one well. It pours distributively on 24 well plate (Corning) so that it may become, and they are 37 degrees C and 5%CO2 in Cryj2 (1microg/(ml)) about these cells. It cultivated for three days under conditions. Culture supernatant liquid was extracted after culture termination, and cryopreservation was carried out at 20 degrees

C until it used for measurement. The amount of the interleukin 4 contained in culture medium was measured by the commercial measurement kit (Endogen shrine).

[0053] (1) The induction mouse caudal vein of the immunological tolerance by vein administration of a peptide 7 was medicated with the solution of a peptide 7. In the mouse of a control group, it is a phosphate buffer solution (0.01M (pH 7.4)). Vein administration was carried out. Then, according to the above-mentioned approach, immunity of the mouse of both groups was carried out in pernasality by Cryj2. Then, when the antigen presenting cell extracted from the submandibular-lymph-nodes cell and other mice which were extracted from the mouse concerned was cultivated with Cryj2, the growth activity of the lymph gland cell from the mouse which prescribed the peptide 7 for the patient beforehand was falling 29.5% as compared with the control group. Thereby, it became clear that there is activity which controls the immune response to Japan cedar allergen in a peptide 7.

[0054] (2) The solution of the T cell peptide 7 was administered orally to the induction mouse of the immunological tolerance by internal use of a peptide 7 4 times according to the above-mentioned approach between two weeks. In the mouse of a control group, it is a phosphate buffer solution (0.01M (pH 7.4)). It administered orally. Then, immunity of the mouse of both groups was carried out in pernasality by Cryj2. Then, the antigen presenting cell extracted from the submandibular-lymph-nodes cell and other mice which were extracted from the mouse concerned was cultivated for three days with Cryj2, and the amount of cytokine in the culture supernatant was measured. Consequently, the amount of the interleukin 4 produced from the lymph gland cell from the mouse which prescribed the peptide 7 for the patient beforehand was falling 49.8% as compared with the control group. Controlling the immune response to Japan cedar allergen was shown by when this prescribes a peptide 7 for the patient in taking orally.

[0055] (3) The induction mouse caudal vein of the immunological tolerance by vein administration of a peptide 8 was medicated with the solution of a peptide 8. In the mouse of a control group, it is a phosphate buffer solution (0.01M (pH 7.4)). Vein administration was carried out. Then, according to the above-mentioned approach, immunity of the mouse of both groups was carried out in pernasality by Cryj2. Then, when the antigen presenting cell extracted from the submandibular-lymph-nodes cell and other mice which were extracted from the mouse concerned was cultivated with Cryj2, the growth activity of the lymph gland cell from the mouse which prescribed the peptide 8 for the patient beforehand was falling 30.9% as compared with the control group. Thereby, it became clear that there is activity which controls the immune response to Japan cedar allergen in a peptide 8.

[0056] (4) The solution of the T cell peptide 8 was administered orally to the induction mouse of the immunological tolerance by internal use of a peptide 8 4 times according to the above-mentioned approach between two weeks. In the mouse of a control group, it is a phosphate buffer solution (0.01M (pH 7.4)). It administered orally. Then, immunity of the mouse of both groups was carried out in pernasality by Cryj2. Then, when the antigen presenting cell extracted from the submandibular-lymph-nodes cell and other mice which were extracted from the mouse concerned was cultivated with Cryj2, the growth activity of the lymph gland cell from the mouse which prescribed the peptide 8 for the patient beforehand was falling 73.1% as compared with the control group. Thereby, it became clear that there is activity which controls the immune response to Japan cedar allergen in a peptide 8.

[0057] About example of trial 4 peptide 8, it treated to the Balb/c mouse. Namely, refined Cryj2 Rhinenchysis administration was carried out and immunity of the antigen solution made to dissolve 1microg and cholera toxin B subunit 1microg (0.5% content of cholera toxins) in 0.01M phosphate buffer solution (pH7.4) was carried out to the Balb/c mouse (5-6 weeks old: Charles RIBAJAPAN) of two groups under the Ava Ching anesthesia. the solution of the peptide 8 dissolved in the 0.01M phosphate buffer solution (pH7.4) from the one-week back to the mouse of an experimental group — one animal — per [200] time It administered orally so that it might become the amount of peptides of mug, and this internal use was repeated 4 times between two weeks. The mouse of a control group was similarly medicated only with the 0.01M phosphate buffer solution (pH7.4). Four days after the 4th internal use, immunity was again carried out to the mouse of both groups in pernasality by Cryj2. After one week, when the submandibular-lymph-nodes cell extracted from the mouse concerned by the same approach as the example 3 of a trial and the antigen presenting cell extracted from other mice were cultivated with Cryj2, growth of the lymph gland cell of the experimental group mouse origin was falling 46.0% as compared with the control group. The peptide 8 became clear [having the activity which controls the immune response to clearance allergen] from this result, also when the mouse after immunity was carried out with clearance allergen was medicated.

[0058] As mentioned above, the peptide of this invention can activate a specific T cell to cedar pollen allergen, without causing anaphylaxis substantially, if the general mammals including Homo sapiens are medicated. If the general mammals including Homo sapiens are medicated with the anti-hay fever agent of this invention which comes to contain this peptide as an active principle, it will demonstrate remarkable therapy and preventive effect to hay fever, without causing anaphylaxis substantially.

[0059] The anti-hay fever agent which comes to contain the peptide of this invention as an active principle can treat hay fever, without causing side effects, such as anaphylaxis, substantially, if the general mammals which is suffered from hay fever and contains Homo sapiens are medicated. When medicating a healthy individual and the individual of potential hay fever with the anti-hay fever agent of this invention before cedar pollen begins to disperse, while demonstrating a remarkable preventive effect to hay fever on the other hand, higher efficacy is demonstrated to the remission of the allergy symptom at the time of the onset.
[0060]
[Embodiment of the Invention] Hereafter, although an example and the example of pharmaceutical preparation explain this invention to a detail further, as for this invention, the technical range is not limited by these. Chemosynthesis of the peptide was carried out by the approach (solid phase synthesis method) of combining amino acid at a time with one amino acid derivative fixed to example 1 peptide 1:Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser resin from a carboxyl-terminus side. The amino acid used in each cycle used the special amino acid derivative with which alpha amino group and the reaction radical of a residue part were blocked by the protective group. Here, each alpha amino group Fmoc (9-fluorenyl methyloxycarbonyl) The amino acid blocked was used (Fmoc law). Moreover, peptide synthesis is alpha amino group of the amino acid combined with resin. Fmoc Deprotection was carried out, and the reaction of combining the amino acid derivative which the carboxyl group activated next was repeated successively, and was performed.
[0061] Each peptide used for an experiment is a multi-peptide synthesizer. SYMPHONY (Protein Technologies, Inc.) is used and it is the above. Fmoc According to the protocol of this equipment, it compounded with the solid phase synthesis method. that is, the amino acid (Ser) equivalent to the C terminal residue of the peptide to compound is introduced Fmoc-Ser(tBu)-Wang-resin (0.52 mmol/g) 25micromol an equivalent -- amino acid which set to the reaction container of the above-mentioned peptide synthesizer unit, and the 1.25 ml deprotection solution (20% piperidine / Dimethyl formamide (DMF)) was made to react twice for 5 minutes, and has been combined with resin Fmoc Except for a radical. DMF 200mM(s) which are equivalent to the 2nd amino acid with 1.25ml of liquid after 6 times washing during 30 seconds, and from the end side of C Fmoc-Ala/DMF 1.25ml of solutions and 1.25ml (200 mM O-Benzotriazole-N, N, ', N', and -Tetramethyl-Uronium-Hexafluoro phosphate / 400 mM N-methylmorpholine/DMF) of activator solutions of 200mM(s) were added (10 times as many : [as this] 250micromol of theoretical equivalence respectively considerable), and it was made to react at a room temperature for 20 minutes. it generated here Fmoc-Ala-Ser(tBu)-Wang-resin -- DMF 1.25ml -- after 6 times washing during 30 seconds -- again -- Fmoc the deprotection of a radical -- using -- DMF 1.25ml -- after 6 times washing during 30 seconds, and Fmoc-Pro The solution and the activator solution were added and were made to react. Peptide made into the purpose by repeating the same actuation (Fmoc-Lys(Boc)-Val-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-resin) It compounded.
[0062] The amino acid used for composition here is as follows (NISSHINBO INDUSTRIES, INC. make). ( ) Inside expresses the protective group which protects the reaction radical of a residue part. Fmoc-Ala Fmoc-Pro Fmoc-Asn (Trt) Fmoc-Gln (Trt) Fmoc-Tyr (tBu) Fmoc-Ile Fmoc-Gly Fmoc-Asp (OtBu) Fmoc-Val Fmoc-Lys (Boc), peptide synthesizer unit SYMPHONY It used and the chestnut **-JI reaction was performed within equipment.
[0063] first, the above -- 1.25ml of deprotection liquid was made to react to the protection peptide resin (Fmoc-Lys(Boc)-Val-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-resin) which might be compounded like twice for 5 minutes, and deprotection of the amino terminal Fmoc radical was carried out to it. Next, 1.25ml DMF After 6 times washing during 30 seconds, and CH2Cl2 It washes similarly. N2 is sprayed. After the desiccation during 10 minutes, Chestnut **-JI solution () [Trifluoroacetic] acid:Phenol:water: -- Tioanisole:Ethanedithiol =82.5:5:5:5:2.5 [2.5ml] are added, and it reacts at a room temperature for 2 hours -- making (D.) [S.King,] [Int.J.Peptide Protein] Cutting of the peptide from Reg., 36, 255 (1990), and resin and removal of an amino acid side chain protective group were performed, and the peptide (Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser) was obtained.
[0064] This peptide solution was filtered after reaction termination using the filter, and it divided into resin and filtrate. Together with 2.5ml of liquid which furthermore washed resin, it collected to the centrifuging tube. The collected peptide solution was picked out from equipment, the 5ml cold ether was added, and the peptide was settled. Centrifugal [of this] was carried out after cooling for a while, settlings (for [3000rpm] 10 minutes) were collected, it repeated collecting, if the cold ether is added again and it is made to distribute 5 to 6 times, and the peptide was washed.
[0065] The obtained peptide was dried and the rough peptide was obtained (50.5mg). After a rough peptide's dissolving in 10% acetonitrile water solution which contains TFA 0.1%, ODS A column (TSKgel ODS-120T, 21.5mmx30cm: TOSOH CORP. make) is supplied. It develops in 21% acetonitrile which contains TFA 0.1% (a

part for 9ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 31 - 35 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (15.9mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0066] It is a peptide () by the same actuation as the example 2 peptide 2:Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser example 1. [Fmoc-Val-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr] (tBu) -Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-resin is compounded. The chestnut **-JI reaction was performed, the peptide (Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser) was obtained, and these peptide solutions were collected to the centrifuging tube. Then, the peptide was settled and the rough peptide was obtained (55.5mg).

[0067] After a rough peptide's dissolving in 10% acetonitrile water solution which contains TFA 0.1%, ODS A column (TSKgel ODS-120T, 21.5mmx30cm: TOSOH CORP. make) is supplied. It develops in 22% acetonitrile which contains TFA 0.1% (a part for 9ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 26 - 29 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (7.1mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0068] The peptide (Fmoc-Asp(OtBu)-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-resin) was compounded by the same actuation as the example 3 peptide 3:Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser example 1, the chestnut **-JI reaction was performed, the peptide (Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser) was obtained, and these peptide solutions were collected to the centrifuging tube. Then, the peptide was settled and the rough peptide was obtained (47.9mg).

[0069] After a rough peptide's dissolving in 10% acetonitrile water solution which contains TFA 0.1%, ODS A column (TSKgel ODS-120T, 21.5mmx30cm: TOSOH CORP. make) is supplied. It develops in 21% acetonitrile which contains TFA 0.1% (a part for 9ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 25 - 28 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (13.8mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0070] The peptide (Fmoc-Trp-Leu-Gln-(Trt)-Phe-Ala-Lys(Boe)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Wang- resin) was compounded by the same actuation as the example 4 peptide 4:Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly example 1. However, it is in C-terminal-amino-acid resin. Fmoc-Gly-Wang - It is 25micromol about resin (0.50mol **-JI / g). It used fairly. The amino acid used for composition is as follows.

[0071] Fmoc-Met, Fmoc-Leu, Fmoc-Thr (tBu), Fmoc-Phe, Fmoc-Gly, Fmoc-Lys (Boc), Fmoc-Ala, Fmoc-Gln (Trt), Perform a chestnut **-JI reaction by the same actuation as Fmoc-Trp and an example 1, and a peptide (Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly) is obtained. These peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained (63.3mg).

[0072] After a rough peptide's dissolving in 20% acetonitrile water solution which contains TFA 0.1%, ODS A column (TSKgel ODS-120T, 21.5mmx30cm: TOSOH CORP. make) is supplied. It develops in 38% acetonitrile which contains TFA 0.1% (a part for 9ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 25 - 31 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (2.0mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0073] The peptide (Fmoc-Trp-Leu-Gln-(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Wang- resin) was compounded by the same actuation as the example 5 peptide 5:Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met example 1. However, it is in C-terminal-amino-acid resin. Fmoc-Met-Wang - It is 25micromol about resin (0.75 mmol/g). It used fairly. The amino acid used for composition is the same as an example 4. The chestnut **-JI reaction was performed by the same actuation as an example 1, the peptide (Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-) was obtained, these peptide solutions were

collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained (29mg).
[0074] A rough peptide is ODS after dissolving in 20% acetonitrile water solution which contains TFA 0.1%. A column (TSKgel ODS-120T, 21.5mmx30cm: TOSOH CORP. make) is supplied, and it develops in 36% acetonitrile which contains TFA 0.1% (a part for 9ml/of the rates of flow, detection wavelength 220nm). After condensing the fraction by which elution was carried out in 32 - 34 minutes, freeze drying was performed and the target peptide was obtained (1.1mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0075] The peptide
(Fmoc-Trp-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang-resin) was compounded by the same actuation as the example 6 peptide
6:Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu example 1. However, it is in C-terminal-amino-acid resin. Fmoc-Leu-Wang - It is 25micromol about resin (0.69 mmol/g). It used fairly. The amino acid used for composition is the same as an example 4.
[0076] The chestnut **-JI reaction was performed by the same actuation as an example 1, the peptide (Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained (35.6mg). A rough peptide is ODS after dissolving in 20% acetonitrile water solution which contains TFA 0.1%. The column (TSKgel ODS-120T, 21.5mmx30cm: TOSOH CORP. make) was supplied, and it developed in 38% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 26 - 30 minutes, freeze drying was performed and the target peptide was obtained (6.3mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0077] Example 7 peptide 7:His-Phe-Thr-Phe-Lys-Val-Asp-Gly-Ile-Ile-Ala-Ala-Tyr-Gln example 1 publication Fmoc By law, it is a product made from Milligen/Biosearch. 9050 Rough peptide 400mg was obtained using the peptide synthesis machine. The muBONDASPHERE 5micro C18C120 A column (19x150mm) was supplied with the rough peptide after dissolving in a TFA water solution 0.1%, and it developed with 90% acetonitrile solution which contains TFA 0.1% (a part for 5ml/of the rates of flow, detection wavelength of 214nm), and after evaporating the fraction by which elution was carried out in 28 - 29 minutes, freeze drying was performed and the target peptide was obtained (36mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0078] Example 8 peptide 8:Arg-Ala-Glu-Val-Ser-Tyr-Val-His-Val-Asn-Gly-Ala-Lys-Phe example 1 publication Fmoc By law, it is a product made from Milligen/Biosearch. 9050 Rough peptide 550mg was obtained using the peptide synthesis machine. The muBONDASPHERE 5micro C18C120 A column (19x150mm) was supplied with the rough peptide after dissolving in a TFA water solution 0.1%, and it developed with 90% acetonitrile solution which contains TFA 0.1% (a part for 5ml/of the rates of flow, detection wavelength of 214nm), and after evaporating the fraction by which elution was carried out in 26 - 27 minutes, freeze drying was performed and the target peptide was obtained (60mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0079] Chemosynthesis of the peptide was carried out by the approach (solid phase synthesis method) of combining amino acid at a time with one amino acid derivative fixed to example 9 peptide
9:Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser resin from a carboxyl-terminus side. The amino acid used in each cycle used the special amino acid derivative with which alpha amino group and the reaction radical of a residue part were blocked by the protective group. Here, each alpha amino group Fmoc (9-fluorenyl methyloxycarbonyl) The amino acid blocked was used (Fmoc law). Moreover, peptide synthesis is alpha amino group of the amino acid combined with resin. Fmoc Deprotection was carried out, and the reaction of combining the amino acid derivative which the carboxyl group activated next was repeated successively, and was performed.
[0080] Each peptide used for an experiment is a multi-peptide synthesizer. SYMPHONY (Protein Technologies, Inc.) is used and it is the above. Fmoc According to the protocol of this equipment, it compounded with the solid phase synthesis method. that is, the amino acid (Ser) equivalent to the C terminal residue of the peptide to compound is introduced Fmoc-Ser(tBu)-Wang-resin (0.52 mmol/g) 25micromol an equivalent -- amino acid which set to the reaction container of the above-mentioned peptide synthesizer unit, and the 1.25 ml deprotection solution (20% piperidine / Dimethyl formamide (DMF)) was made to react twice for 5 minutes, and has been combined with resin Fmoc Except for a radical. DMF 200mM(s) which are equivalent to the 2nd

amino acid with 1.25ml of liquid after 6 times washing during 30 seconds, and from the end side of C Fmoc-Ala/DMF 1.25ml of solutions and 1.25ml (200 mM O-Benzotriazole-N, N, ', N', and -Tetramethyl-Uronium-Hexafluoro phosphate / 400 mM N-methylmorpholine/DMF) of activator solutions of 200mM(s) were added (10 times as many : [as this] 250micromol of theoretical equivalence respectively considerable), and it was made to react at a room temperature for 20 minutes. it generated here Fmoc-Ala-Ser(tBu)-Wang-resin -- DMF 1.25ml — after 6 times washing during 30 seconds -- again — Fmoc the deprotection of a radical — using — DMF 1.25ml — after 6 times washing during 30 seconds, and Fmoc-Pro The solution and the activator solution were added and were made to react. Peptide made into the purpose by repeating the same actuation
(Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang-resin) It compounded.
[0081] The amino acid used for composition here is as follows (NISSHINBO INDUSTRIES, INC. make). ( ) Inside expresses the protective group which protects the reaction radical of a residue part.
Fmoc-Ala Fmoc-Pro Fmoc-Asn (Trt) Fmoc-Gln (Trt) Fmoc-Tyr (tBu) Fmoc-Ile Fmoc-Gly, peptide synthesizer unit SYMPHONY It used and the chestnut **-JI reaction was performed within equipment.
[0082] first, the above — 1.25ml of deprotection liquid was made to react to the protection peptide resin (Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Wang- resin) which might be compounded like twice for 5 minutes, and deprotection of the amino terminal Fmoc radical was carried out to it. Next, 1.25ml DMF After 6 times washing during 30 seconds, and CH2Cl2 It washes similarly. N2 is sprayed. After the desiccation during 10 minutes, Chestnut **-JI solution () [Trifluoroacetic] acid:Phenol:water: -- Tioanisole:Ethanedithiol =82.5:5:5:5:2.5 [2.5ml] are added, and it reacts at a room temperature for 2 hours -- making (D.) [S.King,] [Int.J.Peptide Protein] Cutting of the peptide from Reg., 36, 255 (1990), and resin and removal of an amino acid side chain protective group were performed, and the peptide (Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser) was obtained.
[0083] This peptide solution was filtered after reaction termination using the filter, and it divided into resin and filtrate. Together with 2.5ml of liquid which furthermore washed resin, it collected to the centrifuging tube. The collected peptide solution was picked out from equipment, the 5ml cold ether was added, and the peptide was settled. Centrifugal [of this] was carried out after cooling for a while, settlings (for [3000rpm] 10 minutes) were collected, it repeated collecting, if the cold ether is added again and it is made to distribute 5 to 6 times, and the peptide was washed.
[0084] The obtained peptide was dried and the rough peptide was obtained. Among the obtained rough peptides, after dissolving 11mg in 2ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 3 times and is ODS. A column (TSKgel ODS-120T, 7.8mmx30cm: TOSOH CORP. make) is supplied. It develops in 21% acetonitrile which contains TFA 0.1% (a part for 2ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 9.2 - 11 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (5mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0085] The peptide
(Fmoc-Gly-Ile-Ile-Ala-Ala-Tyr(tBu)-Gln(Trt)-Asn(Trt)-Pro-Ala-Ser(tBu)-Trp-Wang-resin) was compounded by the same actuation as the example 10 peptide 10:Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp example 9. However, it is in C-terminal-amino-acid resin. Fmoc-Trp-Wang - It is 25micromol about resin (0.66 mmol/g). It used fairly. The amino acid used for composition is as follows.
[0086]
Fmoc-Ala, Fmoc-Pro, Fmoc-Asn (Trt), Fmoc-Gln (Trt), Fmoc-Tyr (tBu), Fmoc-Ile, Fmoc-Gly, Perform a chestnut **-JI reaction by the same actuation as the Fmoc-Ser (tBu) example 9, and a peptide (Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp) is obtained. These peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.
[0087] Among the obtained rough peptides, after dissolving 9mg in 4ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 23% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 32 - 38 minutes, freeze drying was performed and the target peptide was obtained (2.5mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0088] The peptide
(Fmoc-Ile-Trp-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang-resin) was compounded by the same actuation as the example 11 peptide
11:Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu example 9. However, it is in

C-terminal-amino-acid resin. Fmoc-Leu-Wang - It is 25micromol about resin (0.69 mmol/g). It used fairly. The amino acid used for composition is as follows.

[0089]

Fmoc-Leu, Fmoc-Thr (tBu), Fmoc-Phe, Fmoc-Gly, Fmoc-Lys (Boc), Fmoc-Ala, Fmoc-Gln (Trt), Fmoc-Trp, Fmoc-Ile, perform a chestnut **-JI reaction by the same actuation as an example 9, and a peptide (Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu) is obtained. These peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.

[0090] Among the obtained rough peptides, after dissolving 7mg in 4ml 20% acetonitrile water solution which contains TFA 0.1%, It divides into 3 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It develops in 37% acetonitrile which contains TFA 0.1% (a part for 2ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 17 - 20 minutes was isolated preparatively, freeze drying after concentration was performed, and the target peptide was obtained. (0.7mg) . This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0091] The peptide (Fmoc-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang-resin) was compounded by the same actuation as the example 12 peptide 12:Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu example 9, the chestnut **-JI reaction was performed, the peptide (Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu) was obtained, and these peptide solutions were collected to the centrifuging tube. Then, the peptide was settled and the rough peptide was obtained. The amino acid used for composition is as follows.

[0092]

Fmoc-Leu, Fmoc-Thr (tBu), Fmoc-Phe, Fmoc-Gly, Fmoc-Lys (Boc), Fmoc-Ala, Inside of Fmoc-Gln (Trt) and the obtained rough peptide After dissolving 9.6mg in 2ml 20% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It develops in 32% acetonitrile which contains TFA 0.1% (a part for 2ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 11 - 16 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (6.4mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0093] The peptide (Fmoc-Leu-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Wang-resin) was compounded by the same actuation as the example 13 peptide 13 Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met example 9. However, it is in C-terminal-amino-acid resin. Fmoc-Met-Wang - It is 25micromol about resin (0.75 mmol/g). It used fairly. The amino acid used for composition is the same as an example 12. The chestnut **-JI reaction was performed by the same actuation as an example 9, the peptide (Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.

[0094] Among the obtained rough peptides, after dissolving 8mg in 2ml 20% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 30% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 25 - 32 minutes, freeze drying was performed and the target peptide was obtained (1.1mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0095] The peptide (Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Wang-resin) was compounded by the same actuation as the example 14 peptide 14:Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu example 9. However, it is in C-terminal-amino-acid resin. Fmoc-Leu-Wang - It is 25micromol about resin (0.69 mmol/g). It used fairly. The amino acid used for composition is the same as an example 12. The chestnut **-JI reaction was performed by the same actuation as an example 9, the peptide (Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.

[0096] Inside of the obtained rough peptide After dissolving 2.5mg in 1ml 20% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 30% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 10 - 12 minutes, freeze drying was performed and the target

peptide was obtained (0.6mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0097] Example 15 peptide 15 : The peptide (Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Wang-resin) was compounded by the same actuation as the Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met example 9. However, it is in C-terminal-amino-acid resin. Fmoc-Met-Wang - It is 25micromol about resin (0.75 mmol/g). It used fairly. The amino acid used for composition is the same as an example 12. The chestnut **-JI reaction was performed by the same actuation as an example 9, the peptide (Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.

[0098] Among the obtained rough peptides, after dissolving 7mg in 4ml 20% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 30% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 15 - 20 minutes, freeze drying was performed and the target peptide was obtained (1.9mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0099] The peptide (Fmoc-Gln(Trt)-Phe-Ala-Lys(Boc)-Leu-Thr(tBu)-Gly-Phe-Thr(tBu)-Leu-Met-Gly-Wang-resin) was compounded by the same actuation as the example 16 peptide 16:Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly example 9. However, it is in C-terminal-amino-acid resin. Fmoc-Gly-Wang - It is 25micromol about resin (0.50 mmol/g). It used fairly. The amino acid used for composition is as follows.

[0100]

Fmoc-Leu Fmoc-Thr (tBu) Fmoc-Phe, Fmoc-Gly Fmoc-Lys (Boc) Fmoc-Ala, Fmoc-Gln (Trt) The chestnut **-JI reaction was performed by the same actuation as the Fmoc-Met example 9, the peptide (Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.

[0101] Among the obtained rough peptides, after dissolving 13mg in 6ml 20% acetonitrile water solution which contains TFA 0.1%, It divides into 3 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 29% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 17 - 20 minutes, freeze drying was performed and the target peptide was obtained (0.9mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0102] The peptide (Fmoc-Ile-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Wang- resin) was compounded by the same actuation as the example 17 peptide 17:Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn example 9. However, it is in C-terminal-amino-acid resin. It is 25micromol about Fmoc-Asn(Trt)-Wang-resin (0.60 mmol/g). It used fairly. The amino acid used for composition is as follows.

[0103]

Fmoc-Leu, Fmoc-Asn (Trt), Fmoc-Ile, Fmoc-Phe, Fmoc-Lys (Boc), Fmoc-His (Trt), Fmoc-Ala, Fmoc-Gln (Trt), Perform a chestnut **-JI reaction by the same actuation as Fmoc-Ser (tBu) and an example 9, and a peptide (Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn) is obtained. These peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.

[0104] Inside of the obtained rough peptide After dissolving 3.8mg in 4ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It develops in 18% acetonitrile which contains TFA 0.1% (a part for 2ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 12 - 15 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (1.9mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.

[0105] Example 18 peptide 18 :P The peptide (Fmoc-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn(Trt)-Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Thr(tBu)-Wang-re was compounded by the same actuation as the he-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr example 9. However, it is in C-terminal-amino-acid resin. It is 25micromol about Fmoc-Thr(tBu)-Wang-resin

(0.50 mmol/g). It used fairly. The amino acid used for composition is as follows.
[0106]
Fmoc-Leu Fmoc-Asn (Trt) Fmoc-Phe, Fmoc-Lys (Boc) Fmoc-His (Trt) Fmoc-Ala, Fmoc-Gln (Trt) The chestnut **-JI reaction was performed by the same actuation as Fmoc-Ser (tBu) and an example 9, the peptide (Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.
[0107] Among the obtained rough peptides, after dissolving 5mg in 4ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 15% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 22 - 30 minutes, freeze drying was performed and the target peptide was obtained (3.5mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0108] It is a peptide () by the same actuation as the example 19 peptide
19-he-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn example 9. [Fmoc-Phe-Ala-Ser(tBu)-Lys(Boc)-Asn] (Trt) -Phe-His(Trt)-Leu-Gln(Trt)-Lys(Boc)-Asn(Trt)-Wang - Resin is compounded. The chestnut **-JI reaction was performed, the peptide (Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn) was obtained, and these peptide solutions were collected to the centrifuging tube. Then, the peptide was settled and the rough peptide was obtained. The amino acid used for composition is the same as an example 18.
[0109] Among the obtained rough peptides, after dissolving 6mg in 4ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 15% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 20 - 28 minutes, freeze drying was performed and the target peptide was obtained (3.8mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0110] The peptide
(Fmoc-Leu-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Wang-resin) was compounded by the same actuation as the example 20 peptide
20:Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu example 9. However, it is in C-terminal-amino-acid resin. Fmoc-Leu-Wang - Resin (0.69 mmol/g) was used by 25micromol. The amino acid used for composition is as follows.
[0111]
Fmoc-Leu, Fmoc-Thr (tBu), Fmoc-Asn (Trt), Fmoc-Gly, Fmoc-Lys (Boc), Fmoc-Cys (Trt), Fmoc-Ala, Fmoc-Ser (tBu), Perform a chestnut **-JI reaction by the same actuation as the Fmoc-Ile example 9, and a peptide (Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu) is obtained. These peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.
[0112] Inside of the obtained rough peptide 10mg After dissolving in 4ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 3 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It develops in 23% acetonitrile which contains TFA 0.1% (a part for 2ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 18 - 22 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (0.9mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0113] The peptide
(Fmoc-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Wang- resin) was compounded by the same actuation as the example 21 peptide
21:Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu example 9, the chestnut **-JI reaction was performed, the peptide (Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu) was obtained, and these peptide solutions were collected to the centrifuging tube. Then, the peptide was settled and the rough peptide was obtained. The amino acid used for composition is the same as an example 20.
[0114] Inside of the obtained rough peptide After dissolving 6.6mg in 2ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It develops in 19% acetonitrile which contains TFA 0.1% (a part for 2ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 17 - 22 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (1.5mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid

sequence shown above was checked.
[0115] The peptide
(Fmoc-Lys(Boc)-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Wang- resin) was compounded by the same actuation as the example 22 peptide
22:Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn example 1. However, it is in C-terminal-amino-acid resin. It is 25micromol about Fmoc-Asn(Trt)-Wang-resin (0.60 mmol/g). It used fairly. The amino acid used for composition is the same as an example 20. The chestnut **-JI reaction was performed by the same actuation as an example 9, the peptide (Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.
[0116] Inside of the obtained rough peptide After dissolving 6.9mg in 1ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 2 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 22% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 9 - 12 minutes, freeze drying was performed and the target peptide was obtained (1.6mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0117] The peptide
(Fmoc-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Wang-resin) was compounded by the same actuation as the example 23 peptide
23:Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn example 9, the chestnut **-JI reaction was performed, the peptide (Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn) was obtained, and these peptide solutions were collected to the centrifuging tube. Then, the peptide was settled and the rough peptide was obtained. The amino acid used for composition is as follows.
[0118]
Fmoc-Leu, Fmoc-Thr (tBu), Fmoc-Gly, Fmoc-Cys (Trt) Fmoc-Ala, Among Fmoc-Ser (tBu) and the rough peptide obtained Fmoc-Ile, after dissolving 6mg in 1ml 20% acetonitrile water solution which contains TFA 0.1%, It divides into 3 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It develops in 19% acetonitrile which contains TFA 0.1% (a part for 2ml/of the rates of flow, detection wavelength 220nm). The fraction by which elution was carried out in 15 - 17 minutes was isolated preparatively, after concentration, freeze drying was performed and the target peptide was obtained (0.9 mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0119] The peptide
(Fmoc-Leu-Thr(tBu)-Ser(tBu)-Gly-Lys(Boc)-Ile-Ala-Ser(tBu)-Cys(Trt)-Leu-Asn(Trt)-Asp(OtBu)-Wang-resin) was compounded by the same actuation as the example 24 peptide
24:Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp example 9. However, it is in C-terminal-amino-acid resin. Fmoc-Asp(OtBu)-Wang - It is 25micromol about resin (0.42 mmol/g). It used fairly. The amino acid used for composition is as follows.
[0120]
Fmoc-Leu Fmoc-Thr (tBu) Fmoc-Gly, Fmoc-Cys (Trt) Fmoc-Ala Fmoc-Ser (tBu), Fmoc-Ile The chestnut **-JI reaction was performed by the same actuation as the Fmoc-Asn (Trt) example 9, the peptide (Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp) was obtained, these peptide solutions were collected to the centrifuging tube, the peptide was settled after that, and the rough peptide was obtained.
[0121] Inside of the obtained rough peptide After dissolving 7.5mg in 1ml 10% acetonitrile water solution which contains TFA 0.1%, It divides into 3 times and is ODS. A column (TSKgel ODS-120T, 7.8mm x30cm: TOSOH CORP. make) is supplied. It developed in 18% acetonitrile which contains TFA 0.1%, and after condensing the fraction by which elution was carried out in 17 - 19 minutes, freeze drying was performed and the target peptide was obtained (0.6mg). This compound peptide About 50 pmol, it is an amino acid sequence analyzer. PPSQ-10 mold (product made from Shimadzu Corp.) When it used and amino acid sequence analysis was performed, the amino acid sequence shown above was checked.
[0122] The example 1 of pharmaceutical preparation
It is 1% (w/v) as a stabilizer so that it may become the last concentration of 0.1g/ml about either of 24 kinds of peptides obtained by the approach the liquids-and-solutions example 1 thru/or given in 24. It dissolved in distilled water containing purified gelatin, sterilization filtration was carried out with the conventional method, and 24 kinds of liquids and solutions were obtained.
[0123] Since usually changes for every individual, this article uses the susceptibility over the peptide of this invention for 24 kinds of liquids and solutions, blending suitably so that it may become the presentation which

was most suitable for each individual. Since this article is excellent in stability, it is useful as liquids and solutions for the ophthalmic solutions for treating and preventing hay fever, a nasal drop, and the sprays in the oral cavity.

[0124] The example 2 of pharmaceutical preparation

as an injections stabilizer — 1% (w/v) 24 kinds of peptides obtained by the approach an example 1 thru/or given in 24 to the physiological saline containing a human serum albumin — respectively — last concentration 0. — 01, 0.1, or 1mg/ml After dissolving and carrying out sterilization filtration so that it may become, 2ml was poured distributively into each sterilization vial bottle, and it freeze-dried and sealed into it.

[0125] In advance of administration, first, this article adds 1ml of distilled water for injection etc. in a vial bottle, and, subsequently to homogeneity, dissolves and uses contents. This article which is excellent in stability and comes to contain 24 kinds of polypeptides by this invention as an active principle is useful as desiccation pharmaceutical preparation for treating and preventing hay fever.

[0126] The example 3 of pharmaceutical preparation

Purification with a tablet average molecular weight of about 20,000dalton pullulan 2g It dissolves in 100ml of distilled water at homogeneity, and is 1.7% of cyanuric chloride (w/v) to a solution. It was made to react at 5 degrees C under stirring for 2 hours, adding 2ml of acetone solutions and a sodium-carbonate water solution maintaining pH at the seven neighborhoods 5% (w/v). Then, keeping pH of a reactant the same to the seven neighborhoods, it dialyzed to 4-degree C cold water overnight, and 20ml of water solutions containing an activation pullulan was obtained.

[0127] It was made to react at 37 degrees C for 12 hours, stirring quietly adding 0.2mg of peptides obtained by the approach an example 1 thru/or given in 24, respectively, and maintaining pH of a solution at the seven neighborhoods. It is 4g about a glycine after a reaction and to a reactant. In addition, stirring quietly, it incubated at 37 degrees C for 5 hours, and the unreacted active group was blocked. A reactant is condensed and it is 0.1M beforehand. Phosphate buffer solution (pH 7.0) Sephadex made to equilibrate G-50 The column was supplied, the same buffer solution fresh to a column was dipped, and the fraction containing the peptide of this invention and the complex of a pullulan was extracted. Yield was about 30% per raw material peptide solid content.

[0128] According to the conventional method, sterilization filtration was carried out, this fraction was condensed, it freeze-dried, the mannitol was mixed to homogeneity after grinding, mixture was tableted, and 2, 10, or the tablet included 50mg was obtained for product 1 lock (200mg) per complex. This article excellent in intake nature and stability is useful as a hypoglottis agent for treating and preventing hay fever.

[0129] The example 4 of pharmaceutical preparation

1g of purification RIBO polysaccharides of the syrups Escherichia coli origin Dissolved in 100ml of 10mM calcium phosphate solutions, added 6ml of 100mM sodium periodate to the solution, it was made to react for 20 minutes under a room temperature, and the RIBO polysaccharide was activated. It is 1M [4-degree C] about a reactant. Glycine-hydrochloric-acid buffer solution (pH 4.4) After receiving, dialyzing overnight and removing unreacted periodic acid, 0.1M While the sodium-hydrogencarbonate buffer solution adjusts to the pH 9.5 neighborhood Separately, it is 0.1M about 24 kinds of peptides obtained by the approach an example 1 thru/or given in 24. 10mg dissolves at a time in a 100 ml phosphate buffer solution (pH 7.0), respectively, and it put for 12 hours and was made to react under a room temperature in addition to the above-mentioned reactant containing an activation RIBO polysaccharide.

[0130] Then, the fraction which refines the newly obtained reactant by the approach of the example 3 of pharmaceutical preparation, and contains the peptide of this invention and the complex of a RIBO polysaccharide which were obtained was condensed, and it freeze-dried, and it ground and considered as the solid state material. Yield was about 30% per raw material peptide solid content. The last concentration is 0.1 or 1mg/ml about sucrose in this solid, respectively. Or 50% (w/w) It is purified gelatin as a stabilizer so that it may become 1% (w/w) It dissolved in included distilled water, sterilization filtration of the solution was carried out with the conventional method, and the sirupy object was obtained. It poured distributively and sealed 2ml of this sirupy object at a time into the sterilization vial bottle, and considered as the product. This article which is excellent in stability and contains the peptide of this invention and the complex of a RIBO polysaccharide as an active principle is useful as syrups for treating and preventing hay fever.

[0131]

[Effect of the Invention] The anti-hay fever agent which comes to contain the peptide and them which consist only of a T cell epitope of cedar pollen allergen by this invention as an active principle was able to be offered. And the peptide of this invention can activate a specific T cell to cedar pollen allergen, without causing anaphylaxis substantially, if the general mammals including Homo sapiens are medicated since it does not react to an immunoglobulin E antibody specific to cedar pollen allergen substantially.

[0132]

[Layout Table]

array number: — die-length [of one array]: — mold [of 14 arrays]: — amino acid topology: — class [of straight chain-like array]: — peptide array Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser 1 5 10 [0133] array number: — die-length [of two arrays]: — mold [of 13 arrays]: — amino acid topology: — class [of straight chain-like array]: — peptide array Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser 1 5 10 [0134] array number: — die-length [of three arrays]: — mold [of 12 arrays]: — amino acid topology: —

配列

Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser

1 5 10

class [of straight chain-like array]: — a peptide

[0135] array number: — die-length [of four arrays]: — mold [of 14 arrays]: — amino acid topology: — class [of straight chain-like array]: — peptide array Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly 1 5 10 [0136] array number: — die-length [of five arrays]: — mold [of 13 arrays]: — amino acid topology: — class [of straight chain-like array]: — peptide array Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met 1 5 10 [0137] array number: — die-length [of six arrays]: — mold [of 12 arrays]: — amino acid topology: —

配列

Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu

1 5 10

class [of straight chain-like array]: — a peptide

[0138] array number: — die-length [of seven arrays]: — mold [of 14 arrays]: — amino acid topology: — class [of straight chain-like array]: — peptide array His Phe Thr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln 1 5 10 [0139] array number: — die-length [of eight arrays]: — mold [of 14 arrays]: — amino acid topology: — class [of straight chain-like array]: — peptide array Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe 1 5 10 [0140] array number: — die-length [of nine arrays]: — mold [of 11 arrays]: — amino acid topology:

配列

Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser

1 5 10

— class [of straight chain-like array]: — a peptide

[0141] array number: — die-length [of ten arrays]: — mold [of 12 arrays]: — amino acid topology: — class

配列

Gly-Ile-Ile-Ala-Ala-Tyr-Gln-Asn-Pro-Ala-Ser-Trp

[of straight chain-like array]: — a peptide 1 5 10

[0142] array number: — die-length [of 11 arrays]: — mold [of 13 arrays]: — amino acid topology: — class [of straight chain-like array]: — peptide array Ile-Trp-Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu 1 5 10 [0143] array number: — die-length [of 12 arrays]: — mold [of 11 arrays]: — amino acid topology: —

配列

Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu

1 5 10

class [of straight chain-like array]: — a peptide

[0144] array number: — die-length [of 13 arrays]: — mold [of 12 arrays]: — amino acid topology: — class [

配列

Leu-Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met

of straight chain-like array]: — a peptide 1 5 10

[0145] array number: — die-length [of 14 arrays]: — mold [of ten arrays]: — amino acid topology: — class

配列

Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu

[of straight chain-like array]: — a peptide 1 5 10

[0146] array number: — die-length [of 15 arrays]: — mold [of 11 arrays]: — amino acid topology: — class [

配列

Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met

of straight chain-like array]: — a peptide 1 5 10

[0147] array number: — die-length [of 16 arrays]: — mold [of 12 arrays]: — amino acid topology: — class [

配列
Gln-Phe-Ala-Lys-Leu-Thr-Gly-Phe-Thr-Leu-Met-Gly
of straight chain-like array]: -- a peptide 1 5 10

[0148] array number: -- die-length [of 17 arrays]: -- mold [of 12 arrays]: -- amino acid topology: -- class [
配列
Ile-Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn
of straight chain-like array]: -- a peptide 1 5 10

[0149] array number: -- die-length [of 18 arrays]: -- mold [of 12 arrays]: -- amino acid topology: -- class [
配列
Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn-Thr
of straight chain-like array]: -- a peptide 1 5 10

[0150] array number: -- die-length [of 19 arrays]: -- mold [of 11 arrays]: -- amino acid topology: -- class [
配列
Phe-Ala-Ser-Lys-Asn-Phe-His-Leu-Gln-Lys-Asn
of straight chain-like array]: -- a peptide 1 5 10

[0151] array number: -- die-length [of 20 arrays]: -- mold [of 12 arrays]: -- amino acid topology: -- class [
配列
Leu-Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu
of straight chain-like array]: -- a peptide 1 5 10

[0152] array number: -- die-length [of 21 arrays]: -- mold [of 11 arrays]: -- amino acid topology: -- class [
配列
Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu
of straight chain-like array]: -- a peptide 1 5 10

[0153] array number: -- die-length [of 22 arrays]: -- mold [of 12 arrays]: -- amino acid topology: -- class [
配列
Lys-Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn
of straight chain-like array]: -- a peptide 1 5 10

[0154] array number: -- die-length [of 23 arrays]: -- mold [of 11 arrays]: -- amino acid topology: -- class [
配列
Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn
of straight chain-like array]: -- a peptide 1 5 10

[0155] array number: -- die-length [of 24 arrays]: -- mold [of 12 arrays]: -- amino acid topology: -- class [
配列
Leu-Thr-Ser-Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp
of straight chain-like array]: -- a peptide 1 5 10

[Translation done.]